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DISTRIBUTION OF VOLATILE FLAVOR IN GRAPES AND GRAPE JUICES[1]

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INTRODUCTION

A knowledge of the distribution of volatile flavor in grapes and grape products is of value to viticulturists who wish to determine botanical origins, to manufacturers of grape juice who are interested in the cause of deterioration of bottled grape juice during storage, and to food officials who regulate the sale of true and imitation grape products.

Methods have not yet been developed for the determination of all the volatile flavoring constituents of grapes, the chemical character of which is still for the most part unknown. Exact quantitative methods for anthranilic acid ester, usually referred to as methyl anthranilate, one of the most important volatile constituents of certain varieties of grapes, however, are now available, and the total esters and total free volatile acids can be readily determined by well-known methods. Power and Chesnut (*7*)[2] have shown that the content of anthranilic acid ester in authentic grape juices varies with the variety of grapes. Their data, which were intended to be only approximately quantitative, were expressed in the following terms: ++, strong reaction for methyl anthranilate; +, slight but distinct reaction; − negative result. In a previous publication (*6*) they stated that the largest quantity of methyl anthranilate (anthranilic acid ester) which they had found in the red juices of the Concord type was about 0.001 gm. per 500 c. c. of grape juice, and that the largest quantity in light-colored juices was not more than 0.0001 gm. per 500 c. c. of grape juice. These quantities are equivalent to 2.0 and 0.2 mgm. per liter respectively. Power and Chesnut also suggested that a determination of the presence or absence of methyl anthranilate may have some diagnostic value. Scott (*8*), who employed an exact quantitative method, found that eight brands of Concord juice believed to be pure contained from 0.80 to 1.49 mgm. of the ester per liter, and that four brands of Catawba juice believed to be pure contained from 0.11 to 0.40 mgm. per liter.

The purpose of the investigation reported here was to extend the work of Power and Chesnut, and Scott, by determining the exact quantities of anthranilic acid ester, total esters, and total volatile acids in a large number of varieties of grapes and grape juices and by determining the distribution of these constituents in the skins, pulp, and juice of grapes and in the products of the grape resulting from the commercial manufacture of grape juice.

[1] Received for publication Feb. 27, 1926; issued August, 1926.

[2] Reference is made by number (italic) to "Literature cited," p. 310.

METHODS OF ANALYSIS

Several methods have been proposed for the determination of anthranilic acid ester in essential oils, fruit juices, and commercial flavors. Most of them are based on Erdmann's method, which depends upon diazotization and the formation of an azo dye. Erdmann (*3*), and Erdmann and Erdmann (*2, p. 1216*), coupled β-naphthol and also β-naphtholdisulphonic acid with the diazotized anthranilic acid ester. Power (*5*) tested both β-naphthol and dimethylaniline for this purpose, but preferred to use the former because of its greater sensitiveness. Mathewson (*4*) used sodium-α-naphthol-2-sulphonate for the spectrophotometric estimation of aromatic amino compounds, including anthranilic acid ester. Scott (*8*) employed alcoholic α-naphthol solution. The writers' present work was begun before the article by Scott [3] was published. After some preliminary experiments on the methods then available, the writers chose for study Mathewson's procedure, in which hydrazine sulphate is used to destroy the excess of nitrous acid after diazotization. The method which they finally adopted is applied directly to the steam distillate, thus eliminating the extraction of the distillate and the removal of the solvent by evaporation.

DETERMINATION OF ANTHRANILIC ACID ESTER

Apparatus

The apparatus used consisted of the following: (1) A steam generator filled with distilled water (an oil can holding 1 gallon will serve the purpose); (2) a Kjeldahl flask, 750 c. c. capacity, with shortened neck, about 10 inches high over all; (3) a spray tube passing through a rubber stopper and reaching to the bottom of the flask; (4) a Kjeldahl distilling tube; (5) a 10 or 12 inch worm condenser, with tube sealed to the outlet, reaching to the bottom of the receiver; (6) an Erlenmeyer flask, 500 c. c. capacity, to be used as a receiver; (7) tinfoil to cover the rubber stoppers to prevent absorption of the esters; (8) a water bath.

Reagents

The following reagents were used: (1) N hydrochloric acid; (2) sodium nitrite solution (2 per cent); (3) hydrazine sulphate solution, saturated (about 3 gm. per 100 c. c.); (4) sodium-1-naphthol-2-sulphonate (5 per cent solution); (5) sodium carbonate (25 per cent solution); (6) standard solution of methyl anthranilate containing 1 mgm. of methyl anthranilate per 1 c. c., obtained by dissolving 0.25 gm. of methyl anthranilate in 60 c. c. of 95 per cent alcohol and diluting to 250 c. c.

Procedure

Place enough water in the receiving flask (6) to just cover, or seal, the end of the extended condenser tube. Place the sample in the distilling flask (2). If the volume of sample is less than 200 c. c. add water up to this volume. Connect with the condenser (5) and

[3] In this article the statement "3 c. c. of N sodium nitrite," under the heading "Colorimetric Method," 13th line, evidently should be "3 c. c. of N sodium hydroxide."

immerse the flask in the water bath to the level of the contents. When the sample has attained the temperature of the water bath, which should be about boiling, connect with the steam generator and pass a rapid current of steam through the sample for 30 minutes. About 300 c. c. should collect in the receiver during this time. Disconnect the apparatus and wash out the condenser with a little water. Add 25 c. c. of N hydrochloric acid and 2 c. c. of 2 per cent sodium nitrite solution to the distillate. Mix well and allow to stand for exactly 2 minutes. Add 6 c. c. of saturated solution of hydrazine sulphate and mix well for 1 minute, so that the liquid comes in contact with all parts of the flask which may have been touched by the solution when it contained free nitrous acid. Keep the liquid in the flask in rapid motion while quickly adding 5 c. c. of a 5 per cent solution of sodium-α-naphthol-2-sulphonate, and immediately add 15 c. c. of 25 per cent solution of sodium carbonate. The presence of anthranilic acid ester in the distillate is shown by a color ranging from light pink to deep red, the intensity of the color being proportional to the amount of this ester present. The azo dye formed is α-naphtholsulphonic acid diazomethyl anthranilate. Dilute the colored solution to 500 c. c. with distilled water, mix, and compare an aliquot of the solution with a standard or set of standards.

Precautions

In diazotizing 88 mgm. of methyl anthranilate, 2 c. c. of 2 per cent sodium nitrite solution will be used. Enough hydrazine sulphate to destroy completely the excess of nitrous acid present must be added. Otherwise sodium nitrosonaphtholsulphonate, which is intensely yellow and modifies the color of the azo dye, is formed. The excess of this reagent must be kept as small as possible, however, in order to limit the number of side reactions.

The proper volumes of the solutions of sodium-1-naphthol-2-sulphonate and of sodium carbonate should be measured in graduated cylinders when beginning the test. This procedure will enable the operator to add the reagents quickly while keeping the solution in rotation, in such a way that each will become incorporated in the liquid almost immediately. About 5 c. c. of 25 per cent sodium carbonate solution is necessary to neutralize the hydrochloric acid present, but the addition of 15 c. c. of this solution gives a solution containing equal parts of sodium carbonate and sodium bicarbonate, which Mathewson found to have the proper hydrogen-ion concentration to give maximum color for this dye. The red dye formed by methyl anthranilate is gradually saponified in alkaline solution, forming the corresponding compound of anthranilic acid, which is more yellow. For this reason it is necessary to apply the test reaction to standard solutions and distillates at as nearly the same time as possible.

Synthetic samples examined by this procedure gave the results reported in Table 1. The methyl anthranilate used in these experiments consisted of two commercial samples, which were shown to contain 97.1 and 97.7 per cent methyl anthranilate, respectively, when analyzed by Mathewson's spectrophotometric method (*4*).

TABLE 1.—*Anthranilic acid ester in synthetic solutions*

Sample	Ester added	Ester found	Quantity recovered
	Mg	*Mg*	*Per cent*
Water	1.102	1.086	98.6
Do	1.102	1.088	98.8
Do	5.51	5.408	98.2
Do	5.51	5.463	99.2
Do	11.02	10.91	99.0
Do	11.02	10.91	99.0
Do	27.55	26.94	97.8
Do	27.55	26.86	97.5
Grape juice	0.00	0.31	
Do	5.51	5.48	*94.2
Do	11.02	10.90	*96.2
Do	27.55	27.48	*98.6

* Based on quantity of ester added, plus quantity found in the commercial sample of grape juice.

The foregoing method has been used for several years with satisfactory results in the field stations of the Bureau of Chemistry of the United States Department of Agriculture for the analysis of genuine and imitation grape products.

DETERMINATION OF TOTAL VOLATILE ACIDS

Subject 200 to 500 c. c. of grape juice or grape beverage to steam distillation, as described in the method for the determination of anthranilic acid ester. Add phenolphthalein indicator to the distillate, and titrate to a permanent pink color with N/10 alcoholic potash. Report as milligrams of acetic acid per liter (1 c. c. N/10 alkali = 6 mgm. acetic acid).

DETERMINATION OF TOTAL VOLATILE ESTERS

After titration of the volatile acids, add 15 to 25 c. c. excess of N/10 alcoholic potash. Heat under a reflux condenser for 1 to 2 hours. Cool and titrate the excess of alkali with N/10 sulphuric acid. Run a blank saponification, using the same quantity of alcoholic potash in 200 c. c. of water made just alkaline to phenolphthalein. Subtract the number of cubic centimeters of acid required for the sample from that required for the blank, and multiply by 8.8. This will give milligrams of ester in terms of ethyl acetate.

MATERIALS

SOURCE OF SAMPLES

The following samples were obtained through the courtesy of J. S. Caldwell of the Bureau of Plant Industry of the United States Department of Agriculture: Whole grapes—32 varieties, 1 sample each; 13 varieties, 2 samples each; 3 varieties, 3 samples each; 1 variety, 4 samples. Grape juice—3 varieties, 3 samples each; 2 varieties, 4 samples each; 1 variety, 5 samples. In most cases each sample of whole grapes came from a single vine. The samples of grape juice had been stored from 1 to 5 years.

Through the courtesy of a manufacturer of grape juice in Chautauqua County, New York, 5 samples of grape juice, representing 1 variety of grapes, and 1 sample of dried pomace, were obtained. This grape juice had been stored from 1 to 3 years.

The following samples were purchased on the Washington, D. C., market in 1924 and 1925: Whole grapes—5 varieties, 1 sample each; 1 variety, 2 samples; 1 variety, 3 samples; 1 variety, 7 samples. Grape juice—8 brands, 1 sample each; 3 brands, 2 samples each.

Altogether 88 samples of whole grapes, representing 55 varieties, and 41 samples of grape juice, representing 6 varieties and 11 brands, were examined.

PREPARATION OF SAMPLES

In the analysis of the whole grapes, 100 to 500 gm. of stemmed grapes were placed in the distilling flask and crushed with a glass rod (flattened at one end) so that all the skins were broken.

"Hot pressed" juice was obtained for analysis by placing weighed quantities of stemmed grapes in a pan, crushing, and heating to boiling. The boiling was continued for three to five minutes, after which the mixture was transferred to a muslin bag and pressed by hand. Two samples were prepared in this way to determine the effect of heating on the content of volatile flavor. "Cold pressed" juice was obtained by placing weighed quantities of stemmed grapes in a muslin bag and pressing with the aid of a screw press. The juices obtained in this way were analyzed without filtering. Other juices were obtained for analysis by draining the pulp and by pressing or draining the skins which had been carefully separated from the pulp by hand. In several instances the juices obtained from draining the pulp and pressing the skins were mixed for analysis. The little juice which drained off in separating the skins from the pulp was collected and analyzed separately. The pomace, pressed or dried skins, and drained pulp, were analyzed, as was also a sample of commercial dried pomace. The samples of authentic and commercial grape juices, of course, required no special preparation for analysis.

EXPERIMENTAL RESULTS

The quantities of anthranilic acid ester, volatile esters, and volatile acids in 84 samples of whole grapes, representing about 55 varieties, are given in Table 2. Sixty samples, representing about 41 varieties, did not contain a measurable quantity of anthranilic acid ester. The quantities of anthranilic acid ester in the other samples varied from 0.05 to 3.80 mgm. per kilogram. The samples which contained the larger quantities of anthranilic acid ester were more fragrant and more highly flavored than the others, except two samples of the Campbell variety, which contained no anthranilic acid ester but did contain exceptionally large quantities of other volatile esters. The volatile esters appeared to vary directly with the fragrance and flavor of the grapes. The three varieties of the vinifera species (Tokay, Cornochon, and Malaga) did not contain anthranilic acid ester. Power and Chestnut (*7*), who examined two comparatively fresh samples of pasteurized grape juice of this species (Muscat and Petite Sirah), found no anthranilic acid ester. Five additional samples of juice of this species (Burger, Sauvignonvert, Zinfandel, Alicante, and Bouschet) examined by them also gave no reaction for anthranilic acid ester. These data indicate that grapes of the *Vitis vinifera* species do not contain anthranilic acid ester. The one sample of Cunningham variety of whole grapes (*Vitis bourquiniana*) which

was examined contained 0.10 mgm. of anthranilic acid ester per kilogram. Power and Chestnut (7) reported negative results on a sample of grape juice of the species *V. bourquiniana*, variety Herbemont.

TABLE 2.—*Flavor in authentic and commercial samples of whole grapes, 1924 and 1925*

Variety and species [a]	Anthranilic acid ester [b]	Volatile esters [c]	Volatile acids [d]	Anthranilic acid ester in volatile esters [e]	Variety and species [a]	Anthranilic acid ester [b]	Volatile esters [c]	Volatile acids [d]	Anthranilic acid ester in volatile esters [e]
	Milligrams per kilogram			*Per cent*		*Milligrams per kilogram*			*Per cent*
Lutie (L)	0.00	28	37	0.0	Tokay [g] (V)	0.00			0.0
Do	.00	141	35	.0	Cornochon [g] (V)	.00			.0
Alice (L)	.00	57	35	.0	Malaga [g] (V)	.00			.0
Do	.00			.0					
Vergennes (L)	.00	6	3	.0	Diogenes (RL)	*f*.00			.0
					Do	*f*.00			.0
Bertha (L)	.00	84	84	.0	Franklin (RL)	.00			.0
Early Victor (LB)	*f*.00	38	62	.0	Do		46		
Valhallah (LCRVB)	*f*.00			.0	Stray	*f*.00			
Requa (LV)	*f*.00			.0					
Do	*f*.00			.0	Louisiana (B)	.00	8	35	.0
					Do	.00	14	46	.0
Cuyahoga (LV)	*f*.00			.0	Canada (LV)	.00	14	23	.0
Lindley (LV)	*f*.00			.0	Stray	.00	39	16	.0
Jefferson (LV)	*f*.00			.0	Do	.00	113	46	.0
Kingsessing (LV)	*f*.00			.0	Do	.00	15	63	.0
Iona (LV)	.00			.0	Marguerite (LiB)	*f*.00			.0
Do	.00	51	39	.0	Big Hope (LiLV)	.00	174	121	.0
Do	.00	156	59	.0	Cloeta (LiRuLV)	.00	20	48	.0
Do	.00	15	16	.0	Rogers No. 32 (LV)	.00			.0
					Do	.00	37	59	.0
Campbell (LV)	.00	360	70	.0					
Do	.00	366	52	.0	Hartford (LV)	.05			
Rebecca (LV)	.00	51	30	.0	Arkansas (L)	.07			
Do	.00			.0	Cunningham (B)	.10			
Lightfoot (LV)	.00	71	29	.0	Salem [g] (LV)	.12	19	61	.4
					Brighton [g] (LV)	.12	64	71	.1
Essex (LV)	.00	16	3	.0					
Merrimac (LV)	.00	52	33	.0	Etta (RL)	.14			
Mills (LV)	.00	17	8	.0	Rogers No. 32 (LV)	.27	29	35	.5
Do	.00			.0	Cottage (L)	.28	188	62	.1
Salem (LV)	.00			.0	Do	.30	192	53	.1
					Loretto (L)	.30	130	40	.1
Diana (LVA)	.00	48	48	.0					
Do	.00	51	27	.0	Delaware [g] (LBV)	.36			
Brilliant (LVB)	.00			.0	Do	.50			
Do	.00	9	67	.0	Pearl (RL)	.90	6	22	8.8
Do	.00	17	51	.0	Concord (L)	.91			
Brilliant (seed) (LVB)	.00	16	37	.0	Pearl [g] (RL)	.95	7	23	7.9
Bailey (LiLV)	.00			.0	Concord [g] (L)	1.39	57	9	1.4
Mary Favorite (RL)	.00	39	47	.0	Do	1.40	103	53	.8
Do	.00	42	28	.0	Fern Munson (LiLV)	1.40			
Do	.00	50	42	.0	Concord [g] (L)	1.70			
Clinton (RL)	.00	8	7	.0	Niagara [g] (LV)	2.72	46	7	3.4
Do	.00	8	5	.0					
					Concord [g] (L)	3.50	170	50	1.2
Gaertner (VL)	.00			.0	Stray	3.75			
Goethe (VL)	.00	6	37	.0	Concord [g] (L)	3.80	170	50	1.3

[a] L, labrusca; B, bourquiniana; C, candicans; R, riparia; V, vinifera; A, aestivalis; Li, lincecumii; Ru, rupestris.
[b] Expressed as methyl anthranilate.
[c] Expressed as ethyl acetate.
[d] Expressed as acetic acid.
[e] Anthranilic acid ester calculated to ethyl acetate.
[f] Analysis made on 100 to 400-gm. samples; all others on 500-gm. sample.
[g] Commercial samples represented to be of the variety and species indicated; all others were authentic.

Table 3 shows the distribution of flavor as represented by anthranilic acid ester and total esters in different parts of grapes. It

has been generally thought that the volatile flavor of grapes resides almost wholly in the skins. This is not the case, however, as 32, 29, 38, and 44 per cent of anthranilic acid ester, and 56 and 51 per cent of total volatile esters, were found in the drained pulp. It is interesting to note also that one-half or more of the total anthranilic acid ester, and more than one-third of the total volatile esters, remain in the pomace, to be lost unless the pomace is worked over for "second pressing" juice. The sample of commercial pomace which had been dried contained a large quantity of flavor. The largest quantity of anthranilic acid ester which was found was 19.5 milligrams per kilogram of pressed skins of the Concord variety of grapes.

TABLE 3.—*Distribution of flavor in different parts of the grape*

Variety [a] and species [b]	Preparation of sample	Anthranilic acid ester [c]	Volatile esters [d]	Volatile acids [e]	Anthranilic acid ester in volatile esters	Juice, skin, or pulp [f]	Anthranilic acid ester [g]	Volatile esters [g]	Volatile acids [g]
		Mg. per liter	*Mg. per liter*	*Mg. per liter*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Delaware (LBV)	Juice: Pressed from whole grapes (hot).	0.15	70	22	0.1	75	24	57	32
Do	Pressed from whole grapes (cold).	.15	21	18	.4	88	24	64	13
Do	Pressed from skins and drained from pulp.	.18				34	18		
Niagara (LV)	Pressed from whole grapes (hot).	2.50	11	26	13.3	85	44		
Do	Pressed from whole grapes (cold).	2.08	20	11	6.0	90	40		
Do	Pressed from skins and drained from pulp.	2.33				46	48		
Concord (L)	Pressed from skins	4.4	128	53	2.0	33	37	32	30
Do	Drained from pulp	1.6	75	9	1.3	15	6	8	23
Do	Drained from skins and pulp.	1.7	83	32	1.2	31	16	22	17
	Pressed skins, pulp, and seeds:								
Delaware (LBV)	Hot	[h].47	[h]53	[h]46	.5	25	76	43	68
Do	Cold	[h].47	[h]12	[h]119	2.3	12	76	36	87
Niagara (LV)	Hot	[h]3.23				15	56		
Do	Cold	[h]3.07				10	60		
Delaware (LBV)	Pressed skins	[h]2.15				8	50		
Niagara (LV)	do	[h]13.1				4	23		
Concord (L)	do	[h]19.5	[h]127	[h]58	9.0	4	19	4	4
Do	Drained skins	[h]6.0	[h]149	[h]126	2.4	21	40	27	46
Delaware (LBV)	Drained pulp	[h].20				55	32		
Niagara (LV)	do	[h]1.43				44	29		
Concord (L)	do	[h]3.0	[h]154	[h]49	1.1	48	38	50	43
Do	do	[h]3.0	[h]129	[h]48	1.3	47	44	51	37
Do	Commercial dried pomace	[h]16.0	[h]69	[h]28	13.4				

[a] Commercial samples represented as being of the variety indicated.
[b] L, labrusca; B, bourquiniana; V, vinifera.
[c] Expressed as methyl anthranilate.
[d] Expressed as ethyl acetate.
[e] Expressed as acetic acid.
[f] Quantity based on weight of whole grapes.
[g] Based on total quantities in whole grapes.
[h] Milligrams per kilogram.

Data showing change of flavor in grape juice during storage are set forth in Table 4. These data indicate that the content of anthranilic acid ester varies inversely with the period of storage. This is especially noticeable in the case of the Niagara grape juice, no anthranilic acid ester having been found in the sample which had

been stored 5 years, whereas a sample of fresh juice contained 2.08 mgm. of anthranilic acid ester per liter. The low figures for Concord grapes in 1924 may be due to a number of factors. The volatile flavor in grapes is probably subject to seasonal variation, although no data definitely prove it. Caldwell (*1*) has shown that climatic conditions, especially the amount of sunshine during the growing season, markedly affect the total sugar content, total astringency, and titratable acidity of grapes. The fact that the grape-growing season of 1924 in the eastern part of the United States had less sunshine than that of 1919, 1920, 1921, 1922, 1923, and 1925 may explain the low figures reported.

TABLE 4.—*Change in flavor of grape juice during storage*

Variety and species [a]	Crop	Years in storage	Anthranilic acid ester [b]	Volatile esters [c]	Volatile acids [d]
			Milligrams per liter	*Milligrams per liter*	*Milligrams per liter*
Agawam (LV)	1923	1	0.00		
Do	1921	3	.00		
Do	1920	4	.00		
Do	1919	5	.00		
Catawba (LV)	1923	1	.03		
Do	1921	3	.00		
Do	1919	5	.00		
Concord [e] (L)	1925		.80	32	13
Do.[e]	1924	1	.20	26	10
Do.[e]	1924	1	.24	21	8
Do.[e]	1923	1	.50		
Do.[e]	1923	2	.76	39	16
Do.[e]	1922	3	.70	37	15
Do	1921	3	.17		
Do	1920	4	.20		
Do	1919	5	.06		
Delaware (LBV)	1924		.15		
Do	1923	1	.00		
Do	1920	4	.00		
Do	1919	5	.00		
Ives (LA)	1923	1	1.10		
Do	1920	4	.16		
Do	1919	5	.07		
Niagara (LV)	1924		2.08		
Do	1923	1	.68		
Do	1922	2	.55		
Do	1921	3	.18		
Do	1920	4	.12		
Do	1919	5	.00		

[a] L, labrusca; V, vinifera; B, bourquiniana; A, aestivalis.
[b] Expressed as methyl anthranilate.
[c] Expressed as ethyl acetate.
[d] Expressed as acetic acid.
[e] Commercial samples believed to be of the variety indicated. Pressed hot, bottled, and pasteurized. All other samples were authentic, and were pressed cold, bottled, and pasteurized.

The following experiment was conducted to supplement the data which indicated a loss of volatile flavor during storage. A sample of commercial grape juice examined on July 15, 1924, contained 1.35 mgm. of anthranilic acid ester per liter. To this sample was added methyl anthranilate in the proportion of 5.21 mgm. per liter. The sample, which then contained 6.56 mgm. of anthranilic acid ester per liter, was stored for about 10 months. On May 26, 1925, it contained 4.05 mgm. of anthranilic acid ester per liter, a decrease of

2.51 mgm. per liter. It was then stored for a further period of 8 months, at the end of which time (January 29, 1926) it contained only 0.70 mgm. of anthranilic acid ester per liter. The data on volatile esters in Table 4 are not sufficient to warrant any conclusions.

Data on the flavor in 14 samples of commercial bottled grape juices are given in Table 5. The anthranilic acid ester varied from 0.00 to 1.35 mgm. per liter in these samples. The volatile esters in 8 of the samples varied from 5 to 29 mgm. per liter.

TABLE 5.—*Flavor in 14 samples of commercial grape juice*

Sample	Variety and species [a]	Anthranilic acid ester [b]	Volatile esters [c]	Volatile acids [d]	Anthranilic acid ester in total volatile esters [e]
		Milligrams per liter	*Milligrams per liter*	*Milligrams per liter*	*Per cent*
A	?	0.00			0.0
B	Catawba / LV	.00			.0
C	Catawba / LV	.00			.0
D	Concord / L	.16	17	7	.6
E	Concord / L	.16			
E	Concord / L	.20	5	70	2.3
F	Concord / L	.25	5	37	2.9
G	Concord / L	.35	8	43	2.5
H	?	.35	5	69	4.1
I	?	.40	5	99	4.7
J	?	.45	5	97	5.2
H	?	.56			
K	Concord / L	.85	29	12	1.7
G	Concord / L	1.35			

[a] L, labrusca; V, vinifera.
[b] Expressed as methyl anthranilate.
[c] Expressed as ethyl acetate.
[d] Expressed as acetic acid.
[e] Anthranilic acid ester calculated to ethyl acetate.
/ Variety obtained from the bottle labels and therefore not authentic.

CONCLUSIONS

Anthranilic acid ester in grapes, grape products, and imitation grape preparations can be determined quickly and accurately by a new method developed in the Bureau of Chemistry of the United States Department of Agriculture.

The content of anthranilic acid ester, and of total volatile esters is a true measure of the intensity of aroma in grapes and grape juices.

The anthranilic acid ester in 84 samples of whole grapes, representing about 55 varieties, varied from 0.00 to 3.80 mgm. per kilogram. The volatile esters and volatile acids in 50 samples, representing about 34 varieties, varied from 6 to 366 and from 3 to 121 mgm. per kilogram, respectively.

Anthranilic acid ester has not been found in the fruit of *Vitis vinifera*. The determination of this ester, therefore, appears to be of value in identifying species.

Contrary to general opinion, the volatile flavor of grapes is not contained almost wholly in the skins Substantial proportions are found in the pulp. Nearly as much volatile flavor remains in the pomace as is contained in the expressed juices.

Anthranilic acid ester tends to disappear from grape juice which is stored. This fact may explain the deterioration in flavor of certain commercial bottled grape juices.

The anthranilic acid ester in 14 samples of commercial bottled grape juices of unknown origin varied from 0.00 to 1.35 mgm. per liter. The volatile esters in 8 of these samples varied from 5 to 29 mgm. per liter.

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SPECIFIC IDENTITY OF WHIPWORMS FROM SWINE[1]

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INTRODUCTION

Up to the present time it has been generally accepted as an established fact that whipworms occurring in swine represent a distinct species (*Trichuris suis*) that is peculiar to these animals. The doubts expressed by Creplin (*1*)[2] and shared by Leuckart (*4*) concerning the specific identity of swine whipworms appear not to have produced any impression upon parasitologists, who have generally accepted the validity of the name *T. suis*. In the standard textbooks on human and veterinary parasitology the whipworm from hogs (*T. suis*) is differentiated from the form occurring in man (*T. trichiura*), and the possibility of the two species being closely related, if not identical, is entirely overlooked. Furthermore, such well-known treatises on parasitology as Neumann's Treatise on the Parasites of Domesticated Animals (*5*) and Railliet's Traité de Zoologie Médicale et Agricole (*6*), as well as other texts on veterinary parasitology, emphasize the point that the swine whipworm is specifically distinct from the species occurring in man (*Trichuris trichiura*).

However, the present writer's recent studies of whipworms from man, monkeys, and swine have led him to the conclusion that the forms from these hosts are indistinguishable morphologically, and that they therefore represent but a single species, *Trichuris trichiura* Linnaeus, 1758. Schneider (*8*) had already reached the conclusion that whipworms from man and other primates were identical. A study of the literature pertaining to this subject has shown, moreover, that the names *Trichuris suis*, *T. crenatus*, and *T. apri*, all of which are synonyms, were proposed when species of parasites were commonly differentiated on the basis of host relationship, frequently irrespective of morphological organization, and that no valid morphological evidence to differentiate *Trichuris suis* from *T. trichiura* had been given by those who considered the former a valid species. In arriving at the conclusion that swine whipworms are identical with the species occurring in man and in other primates the present writer has been guided solely by morphological relationships. The question concerning the biological relationships of these worms from different hosts and the possibility of cross infection must be left open until experimental tests are performed.

HISTORY OF THE DISCOVERY OF WHIPWORMS IN SWINE

Goeze (*3*) appears to have been the first investigator to record the occurrence of whipworms in swine. He reported two specimens

[1] Received for publication March 5, 1926; issued August, 1926.
[2] Reference is made by number (italic) to "Literature cited," p. 316.

from a wild hog, and although he noted their resemblance to the form occurring in the human he considered them distinct. The specimens recorded by Goeze formed the basis of the name *Trichocephalus suis* Schrank, 1788 (*9*). Schrank did not study Goeze's material, nor does it seem that he studied any other specimens, but merely proposed the name *Trichocephalus suis* on the strength of Goeze's statement that the whipworm from swine is different from the form from humans, which he called *Trichocephalus hominis*. Gmelin (1790) reported whipworms from swine under the name *Trichocephalus apri*, but gave no differentiating characters of the species. Rudolphi (*7*) gave a description of swine whipworms which he called *Trichocephalus crenatus*. As already noted by Creplin (*1*), Rudolphi worked with imperfectly preserved material, so his description very probably was based largely on distorted specimens. Creplin (*1*) appears to have been the first helminthologist to question the

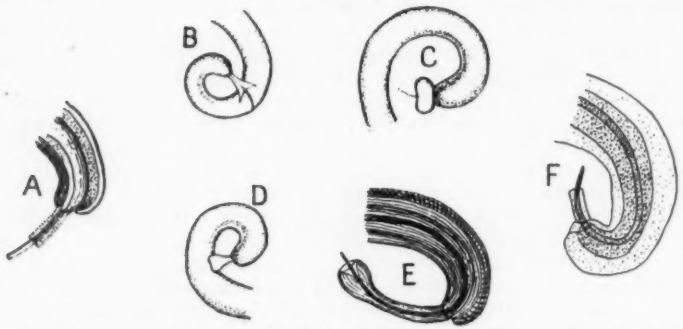

FIG. 1.—Posterior end of Trichuris male. A, Trichuris from man (from Goezl, 1782); B, C, D, Trichuris from swine (from Creplin, 1825); E, Trichuris from man (from Davaine, 1860); F, Trichuris from man (from Urioste, 1923)

validity of the name *Trichocephalus crenatus* (*T. suis*), basing his doubt on his study of specimens from swine which he described and figured. As may be seen from B, C, and D in Figure 1 herein, which are copied from him, Creplin was impressed with the variability of the prepuce-like spicule sheath in swine whipworms, an organ which Schneider (*8*) has made an important differential character between *Trichocephalus crenatus* and *Trichocephalus dispar* (*T. trichiura*). According to Schneider, *Trichocephalus dispar* differs from the swine whipworm, *Trichocephalus crenatus*, not only in the shape of the spicule sheath (fig. 2, L and P), a character that is not mentioned in his text but is emphasized in his illustrations, but also in the relative abundance and shape of the spines on the sheath and the structure of the spicule. Schneider states that the sheath of *Trichocephalus dispar* (fig. 2, L) is thickly covered with pointed spines throughout its length, whereas in *Trichocephalus crenatus* (fig. 2, P) the spines are blunt and scattered and disappear on the posterior part of the sheath. Schneider also differentiates between the spicules in the two species; those of *T. crenatus* terminate bluntly and those of *T. dispar* terminate in a sharp point, according to him. While Schneider's differential characters appear to be sound and have undoubtedly served to weaken opposition to the specific validity of *Trichuris suis*, they seem to have been based on the study of only a few specimens.

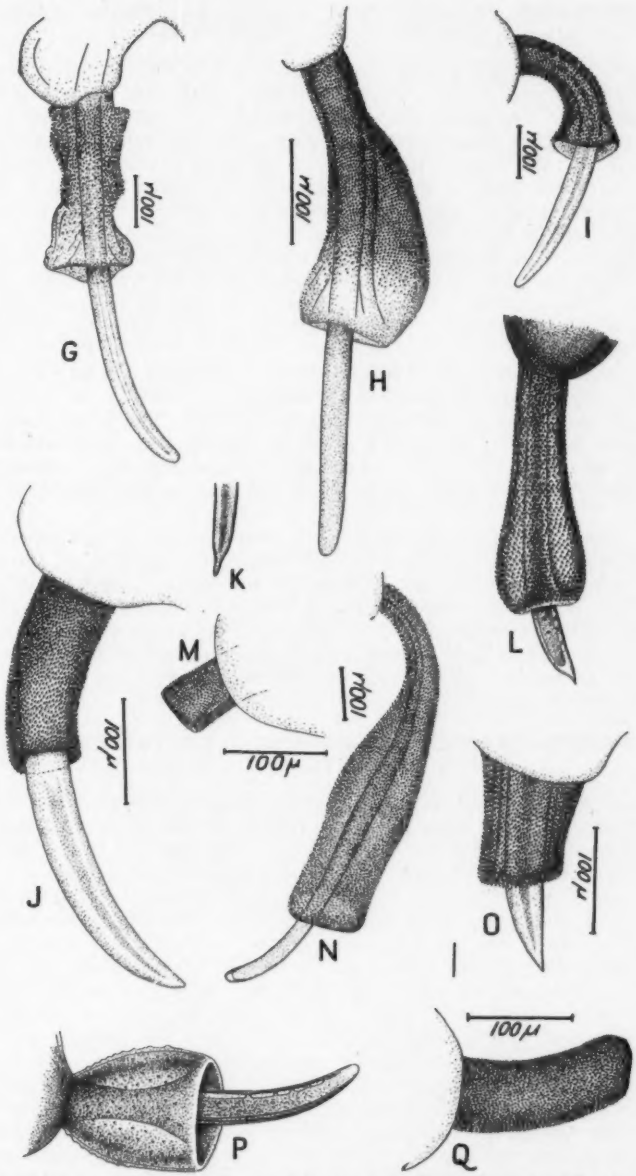

FIG. 2.—Posterior end of Trichuris male. G, H, Trichuris from the chimpanzee (original); I, J, O, Trichuris from swine (original); K, tip of spicule of Trichuris from swine (original); L, Trichuris from man (from Schneider, 1866); M, N, Trichuris from man (original); P, Trichuris from swine (from Schneider, 1866); Q, Trichuris from Cercopithecus (original)

MORPHOLOGY OF TRICHURIS TRICHIURA FROM MAN AND TRICHURIS SUIS FROM SWINE

The specific characters in the genus *Trichuris* Roederer and Wagler, 1701 (Synonym: *Trichocephalus* Schrank, 1788) are largely limited to the size of the spicules, the structure of the sheath, and the size of the eggs. Less important and more variable specific characters include the total size of the worms and the ratio of the slender anterior portion of the body to the relatively thick posterior portion. *Trichuris trichiura* from man, as commonly described in textbooks on parasitology, is from 30 to 50 mm. long, the males being somewhat smaller than the females. The posterior portion of the male is about two-fifths, and that of the female one-third, of the total length of the worm. The spicule is about 2.5 mm. long, and its retractile sheath is covered with spines. The eggs are from 50 to 54 μ long by about 23 μ wide.

The description of *Trichuris suis* in various textbooks on veterinary parasitology gives a variation in length from 33 to 50 mm., the females being somewhat larger than the males. The relatively thick posterior portion of the male is given as three-eighths, and that of the female as one-third, of the total length. According to Railliet, the spicule of *T. crenatus* is 3.35 mm. long and the eggs are from 52 to 56 μ long.

A comparison of the description of these two species indicates their morphological identity, save for the size of the spicule of *Trichuris suis*, which, according to Railliet, is somewhat longer than that recorded for *T. trichiura*. The only other apparently important differential characters of *T. crenatus* appear to be those given by Schneider, who, it may be assumed, found no marked discrepancy in the size of the spicules in the two species, as this character is not mentioned by him.

THE PRESENT WRITER'S OBSERVATIONS ON TRICHURIS FROM MAN, MONKEYS, AND SWINE

Observations by the present writer are based on specimens of Trichuris collected from man in various parts of the world, which were loaned by W. W. Cort, of the School of Hygiene and Public Health of Johns Hopkins University; on specimens from primates other than man, namely, the chimpanzee and Cercopithecus; and on specimens from swine from the United States, the Philippine Islands, and the South Sea Islands. As far as concerns the size of the esophagus (which was found to be about two-thirds of the total length of the body in nearly all forms that were measured), the size of the eggs (which are from 50 to 54 μ long by 25 μ wide in forms from man and swine), and the position of the vulva (which is somewhat posterior to the end of the esophagus in all forms), the specimens from the various hosts are in general agreement with each other and with the available data concerning these characters in whipworms from man and from swine as already given in this paper. As far as the size of the spicules is concerned, no glaring discrepancies were found in specimens from various hosts. The spicule in specimens from the pig varied from 20 to 24 mm. in length; from the chimpanzee, from 20 to 22 mm.; and spicules from specimens from man were about 26.5 mm. long. The tip of the spicule presented marked variations in shape. In specimens from the chimpanzee and from

man (fig. 2, G, H, N) spicules with bluntly rounded tips were found. Similar spicule tips were found in certain specimens from swine (fig. 2, I). Some specimens from swine had gradually tapering spicule tips which terminated in sharp points (fig. 2, O). In one specimen from a pig (fig. 2, K) a spicule tip very similar to that figured by Schneider for the human Trichuris (fig. 2, L) was found.

It is evident from these observations that the shape of the spicule tip is variable in Trichuris from man, monkeys, and swine, and that there is no relation between the shape of the spicule tip and the host from which the worm was obtained, practically the same range of variation occurring in all hosts.

The shape of the sheath presented a similar range of variation, as may be seen by comparing G, H, I, and N of Figure 2, which show practically the same type of sheath in specimens from man, the chimpanzee, and the pig as that figured by Schneider (fig. 2, L) (*8*) as characteristic of the sheath in the human Trichuris. J, M, and Q of Figure 2 show cylindrical sheaths in specimens of Trichuris from the pig, man, and Cercopithecus, respectively. Similar cylindrical sheaths from the human Trichuris have been figured by Goeze (fig. 1, A) (*3*) and by Urioste (fig. 1, F) (*10*). Davaine's figure (*2*) of the spicule sheath of Trichuris from man (fig. 1, E) is very similar in shape to the figure given by Schneider as characteristic of Trichuris from the pig (fig. 2, P).

As far as the abundance and shape of the spines are concerned, the present writer found specimens from the chimpanzee in which the spines were practically absent in the posterior region of the sheath (fig. 2, G, H), a character which Schneider considered specific for the swine whipworm. In a specimen of Trichuris from man (fig. 2, N) the pointed spines in the posterior region of the worm are replaced by blunt scaly spines, whereas in most specimens of swine whipworms examined the entire sheath was thickly covered with pointed spines, the latter a character considered by Schneider as specific to Trichuris from man.

On the basis of these observations it appears that neither the shape of the sheath, nor the abundance and shape of the spines on the posterior part of the sheath, nor the character of the tip of the spicule, is of specific value in differentiating Trichuris from man, monkeys, and swine, because the same degree of variation with respect to these characters occurred in the parasites examined from all the hosts mentioned. The spicule sheath in Trichuris is a retractile organ and apparently capable of considerable expansion and contraction, since the sheath in retracted position is much narrower than when extruded. The abundance and shape of spines may possibly be correlated with the age or with other conditions of different specimens, or these characters are normally exceedingly variable. The observations recorded in this paper are in harmony with the discrepancies in shape of the different figures of the posterior end of the male of Trichuris from man and from swine, and they support the doubts expressed by Creplin (*1*) and Leuckart (*4*) concerning the validity of *T. crenatus* as a species distinct from *T. dispar* (= *T. trichiura*), and they lead the present writer to the conclusion that as far as morphological organization is concerned the swine whipworm belongs to the same species as that occurring in man and in certain other primates. The name *Trichuris suis* must therefore be considered a synonym of *T. trichiura*.

In view of the medical significance of the conclusion that the swine and human whipworms are zoologically indistinguishable, it is highly important to determine whether these worms have developed specific host adaptations, or whether the embryonated eggs of the worms from one host species will be infectious for the other host species. While experiments on man are practically precluded, or at least of doubtful advisability, experiments involving feeding embryonated eggs of human whipworms to swine and of embryonated eggs of swine whipworms to monkeys will be undertaken as soon as the necessary material has been successfully cultured.

SUMMARY

A morphological comparison of whipworms (Trichuris) from man, the chimpanzee, Cercopithecus, and swine, has shown that the worms from these hosts are morphologically indistinguishable, as far as they have been compared. Schneider's differentiation between whipworms from man and other primates and those occuring in swine have been shown to be due to individual variation, since the characters regarded by Schneider as specific for whipworms from primates are present in whipworms from swine, and vice versa. *Trichuris suis* and its synonyms (*T. crenatus* and *T. apri*) must for the present be regarded as synonyms of *Trichuris trichiura*.

Since the probability that the human and swine whipworms are identical implies the possible necessity for taking cognizance of swine as sources of infestation for man, it is important that the possible transmission of *Trichuris* from man to swine and from swine to man, or at least to other primates, be investigated experimentally, and it is hoped to carry out such experiments at an early date.

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LABORATORY EXPERIMENTS WITH ARSENICALS IN THE CONTROL OF THE CODLING MOTH[1]

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INTRODUCTION

It has been the experience of investigators in codling-moth control that orchard-plot tests of various spray materials are not always dependable. Where the difference in results is expected to be small, owing to the variation in the treatment, the difference is often entirely obscured by factors over which the investigator has little or no control. It is well known that natural variations in infestation occur even in small orchards. These may be due to different varieties, to the presence of an adjacent badly infested orchard, or to a fruit packing house. But variations often occur where they would not be expected, and where they can not be forecast.

Several methods have been suggested to overcome these variations. It is possible to spray the whole experimental plot alike the first year, and determine the variation by examining the fruit from a number of trees suitably distributed over the plot. Ball (*1*)[2] recognized the fact that variations occurred, and employed a method of comparing the sprayed trees with adjacent unsprayed trees, giving his results in terms of efficiency rather than in terms of percentage of wormy fruit. Felt (*5*) mentioned a possible relation of the number of "stings" to the total number of wormy apples, and Melander (*10*) suggested employing the ratio of worms to stings to show the results from different treatments. The use of more modern statistical methods may also help to eliminate some of the errors due to natural variations.

Laboratory tests, consisting of feeding sprayed foliage to insects or their larvae, have been conducted by various investigators, notably Tartar and Wilson (*21*), Scott and Siegler (*16*), Lovett (*8*), and Cook and McIndoo (*3*). This method does not seem to have been employed with the codling moth,[3] and it seemed to the writer that many of the uncontrollable factors met with in codling-moth work could be avoided by its use. Accordingly, the writer has made a large number of laboratory tests, consisting essentially of spraying a given number of apples, placing a given number of newly hatched codling-moth larvae on them, and later recording the number of worm holes and stings. This method can be used to show the relative value of various arsenicals, of various dilutions of arsenicals, and of combinations of arsenicals with other spray materials. The ease with which it can be employed enables the investigator to carry out a large number of tests in a single season and to make direct compari-

[1] Received for publication Dec. 30, 1925; issued August, 1926.

[2] Reference is made by number (italic) to "Literature cited," p. 329.

[3] Ralph H. Smith has made a number of tests of this nature, and has presented some of the results in a paper entitled "Experiments on the Efficiency of Lead Arsenate in Protecting Apples against Codling Moth Injury." This paper was read before the meeting of the Pacific Slope Branch of the American Association of Economic Entomologists, June 27, 1924.

sons of a relatively large number of variations in the treatment. Obviously, this method gives only an index of the value of the material used at the time it is applied. It is of no value in determining the durability or adhesiveness of the arsenicals, or of the relative effect of various methods of application (such as by means of the spray gun or rod), or of various numbers of applications, though it has been used to show the difference between light and heavy applications of spray.

The writer has made nearly 300 tests of this nature in 1922, 1923, and 1924, at the fruit-insect investigations laboratory of the United States Bureau of Entomology at Yakima, Wash.

METHODS

After some experimenting, the following method, designed to eliminate experimental error as far as possible, was employed. Only unsprayed apples free from worms were used. Jonathans were used for all of the tests in 1923; Arkansas Blacks were used in 1924, they being available in larger quantities. The calyx was cut from each apple and the wound was filled with melted paraffin, which prevented worms from entering the calyx. The apples were then suspended from strings by their stems in an outdoor shelter. A sticky tree-banding material was put around the strings to prevent stray worms from crawling down to the apples, since large numbers of worms were being reared in the shelter.

The spray was applied with a small hand sprayer, the apples being rotated to insure an even coating. When the apples were thoroughly dry, newly hatched larvae were carefully placed on them by means of a camel's-hair brush. These larvae were obtained from jars in which moths had deposited large numbers of eggs, and they were transferred to the apples as fast as they hatched. Care was taken to use only fresh, active larvae. The larvae available from any one jar were distributed as evenly as possible on all the apples used, in case there should be a difference in the vitality of different lots of larvae. Usually all were placed within 36 hours of applying the spray.

In 1923 three apples were used for each experiment, 50 larvae being placed on the three apples, 16 on one of them and 17 on each of the other two. In 1924 five apples were used and 10 larvae were placed on each. Usually 10 or 12 tests were made at the same time, materials that were to be directly compared being tested. For example, the experiments made one day would include only different strengths of lead arsenate, whereas those made another day would include different arsenicals, or arsenicals with and without other materials. Each test was repeated several times.

In all of the tests of a given material for each year, material from the same container was used, and only one brand of material was used for all of the tests, except where tests of different brands were made. The lead arsenate used in all of the tests was acid lead arsenate.

The worm entrance holes and stings were counted 10 days after the last larvae had been placed on the apples. Blemishes extending not more than one-fourth of an inch into the flesh were considered as stings. A few of the larvae may have produced both a sting and a worm hole, but for the most part the dead larva could be found in the sting, while in the case of the wormhole the larva was still alive and feeding.

RESULTS OF TESTS

In 1922 the tests were of a preliminary nature, and were of value chiefly in determining the best method to employ. The 1923 tests (all made with second-brood larvae) gave some good results, although, owing to a scarcity of larvae part of the time, it was not always possible to make the desired comparisons on the same date. Comparative tests not made on the same date have been eliminated from the tables, as there is danger of error due to differences in the maturity of the apples and to possible differences in the vitality of the larvae.

In 1924 plenty of larvae were available, and it was possible to make the comparative tests on the same dates. For the most part, two tests were made with first-brood larvae, and one test with second-brood larvae.

In presenting the results of these tests in the tables, it has been necessary to give only the summary of all the tests made with any given material or combination of materials. The total number of worms used is not shown, since in each case it is equal to 50 times the number of tests made. The percentage of worms producing wormholes and the percentage producing stings are both recorded.

The more effective treatment is one that not only reduces the number of larvae producing wormholes or total blemishes, but also causes more of the larvae that do produce blemishes to die after producing only a sting. In other words, the more effective treatment is accompanied by more stings than the less effective treatment, but the reduction in the number of wormholes is relatively greater. This production of stings appears to be unavoidable, because rather slow-acting poisons must be used to avoid injury to the fruit or foliage. Fortunately, stung apples are usually worth four or five times as much as wormy apples, though not as much as entirely sound fruit. It was on account of this increase in the relative number of stings with the more effective treatments that Melander (*10*) suggested using the ratio of worms to stings in presenting results of spraying tests. For the same reason the number of stings per wormhole is shown in the tables in this paper, as well as the percentage of larvae producing wormholes and stings. This ratio of stings to wormholes is, as a rule, greater in the more effective than in the less effective treatments. In all of the tests, comparison is made with powdered lead arsenate, diluted to 1 pound to 50 gallons of water, this being considered the standard treatment.

CASEIN SPREADER

Very little has been published to show whether the addition of casein spreaders to insecticides increases their effectiveness. Lovett (*8*), in orchard tests against the codling moth in 1918 and 1919, obtained approximately equal results when he used half-strength lead arsenate with casein spreader, and full-strength lead arsenate without spreader. Stearns and Hough (*19*, *20*), using the standard quantity of lead arsenate, with and without casein spreader, reported no difference in results against the codling moth and other biting insects. Newcomer (*11*) obtained slightly better control of the codling moth with spreader added to the lead arsenate than without. No laboratory tests appear to have been reported.

In 1923 the present writer made a number of laboratory tests with the codling moth, using a prepared casein spreader containing approximately 20 per cent of casein and 80 per cent of hydrated lime. This was added to the standard dilution of powdered lead arsenate at the rate of one-fourth pound and one-half pound to 50 gallons. A test was also made with three applications of the latter strength sprayed on within a few minutes of each other. It was thought that a thicker coat of spray might be obtained in this way. These tests are reported in Table 1, and show a decided improvement in control where the spreader was added. In test 2, with one-fourth pound of spreader, only a few more than half as many larvae entered the fruit as with the standard treatment in test 1, and the number of stings was slightly less. In test 3, with double the amount of spreader, the results were not as good as in test 2, but were better than in test 1. Test 4, sprayed three times, gave approximately the same results as test 3.

In 1924 these tests were repeated, and spreader at the rate of one-eighth pound to 50 gallons was also used. The results reported in Table 2 show that this quantity (test 2) was the best of those tried, and the number of worms entering the fruit was progressively greater as the amount of spreader was increased (tests 3 and 5). In test 4, sprayed three times, noticeably better results were obtained than in test 3, which was sprayed once. All of the tests with spreader gave better results than where no spreader was used.

TABLE 1.—*Comparison of effectiveness of lead arsenate when used with and without casein spreader against the larva of the codling moth, Yakima, Wash., 1923*

Test No.	Material	Number of tests	Number of wormholes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per wormhole
1	Lead arsenate, 1–50; no spreader	4	47	23.5	47	23.5	47.0	1.00
2	Lead arsenate, 1–50; casein spreader, ¼–50	4	25	12.5	35	17.5	30.0	1.40
3	Lead arsenate, 1–50; casein spreader, ½–50	4	28	14.0	53	26.5	40.5	1.89
4	Lead arsenate, 1–50; casein spreader, ½–50; sprayed 3 times	4	30	15.0	47	23.5	38.5	1.57
5	Control (unsprayed)	4	125	62.5	15	7.5	70.0	.12

TABLE 2.—*Comparison of effectiveness of lead arsenate when used with and without casein spreader against the larva of the codling moth, Yakima, Wash., 1924*

Test No.	Material	Number of tests	Number of wormholes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per wormhole
1	Lead arsenate, 1–50; no spreader	3	40	26.7	34	22.7	49.4	0.85
2	Lead arsenate, 1–50; casein spreader, ⅛–50	3	22	14.7	30	20.0	34.7	1.36
3	Lead arsenate, 1–50; casein spreader, ¼–50	3	28	18.7	26	17.3	36.0	.93
4	Lead arsenate, 1–50; casein spreader, ¼–50; sprayed 3 times	3	20	13.3	20	13.3	26.6	1.00
5	Lead arsenate, 1–50; casein spreader, ½–50	3	37	24.7	24	16.0	40.7	.65
6	Control (unsprayed)	3	83	55.3	6	4.0	59.3	.07

Casein spreader was also added to various strengths of lead arsenate and to other arsenicals and spray combinations. The results are noted in the discussions of the respective experiments. The results obtained where casein spreader was used were better than where it was not used, with one exception (tests 8 and 11, Table 11), but in this case it is possible that a reaction occurred between the lime in the spreader and the calcium arsenate, which affected the results.

The addition of casein spreader to the lead arsenate gave a greater improvement in control in these laboratory tests than has been obtained in orchard tests. This suggests that, when freshly applied, the coating of poison with the spreader is much more effective than that without, this being due perhaps to greater uniformity of the coating; but that the coating with spreader is not as durable, due possibly to its being thinner and thus being rendered ineffective more rapidly by the growth of the fruit or by being rubbed off.

After the spreader was added to the spray, better spreading was accomplished when the spray was allowed to stand for about five minutes before using than if it was used immediately.

STRENGTH OF LEAD ARSENATE

Lead arsenate is ordinarily used against the codling moth at the rate of 2 pounds of paste or 1 pound of powder to 50 gallons of water, and this amount has become the standard recommendation. E. D. and W. M. Ball (*1*) obtained an efficiency of 90 per cent with paste lead arsenate used at the rate of 2½ pounds to 50 gallons, and only 80 per cent efficiency with the same material used at the rate of 1¼ pounds to 50 gallons. These tests were made under rather wormy conditions. Melander (*9*), working under favorable conditions, reported very little difference with paste lead arsenate at various strengths from 1–12 to 1–80. De Sellem (*4*) obtained better results with the paste lead arsenate at 2–50 than at 1–50, and equal results with powdered lead arsenate at 1–50 and at ½–50, with a rather light infestation of worms; and Siegler and Plank (*18*), working in a very wormy orchard, apparently obtained better results with powdered lead arsenate at ½–50 than at 1–50. A comparison of the total number of stings with the total number of worms in the latter case, however, is in favor of the larger quantity of lead arsenate. All of these tests were orchard tests, and there is a probability that other factors influenced the results.

Lovett (*8*), in laboratory tests with tent caterpillars, obtained more rapid killing as the strength of paste lead arsenate was increased from ⅛–50 to 2–50, and chemical analyses showed more arsenic oxide (As_2O_5) in the bodies of the caterpillars as the strength was increased.

The present writer made a number of tests with the codling moth, using powdered lead arsenate at various strengths from one-half pound to 3 pounds in 50 gallons of water, both with and without casein spreader. The lead arsenate used was guaranteed to contain 31 per cent of As_2O_5. The tests in 1923 were incomplete, owing to a scarcity of larvae, and only the results from two strengths of lead arsenate are given in Table 3. A complete series of tests was made in 1924, and the results are given in Table 4.

In these tests the number of wormholes was reduced, and the number of stings generally increased as the strength of the lead arsenate was increased. The total number of blemishes was not very greatly reduced by the increase in the amount of poison used. The results obtained with powdered lead arsenate used at a strength of 1–50 and 2–50 are of particular interest, since there is a tendency at the present time to double the amount of material used. Both in 1923 and 1924 (Tables 3 and 4), increasing the amount of lead arsenate from 1–50 to 2–50 reduced the number of wormholes or the total number of blemishes very little. It is possible that the efficiency of the double-strength spray is reduced less rapidly than that of the single-strength as time goes on after it is applied, but this is a point that can not be tested with this type of experimentation. It is not safe to draw conclusions from these tests as to the optimum amount of lead arsenate for orchard use. It should be noted that, with casein spreader added, the double-strength spray resulted in less than half as many wormholes as the single-strength spray (Table 4, tests 8 and 9). In this particular case the number of stings seems to be an exception to the general tendency for the stings to increase as the strength of the spray is increased.

TABLE 3.—*Comparison of the effectiveness of different strengths of powdered lead arsenate when used against the larva of the codling moth, Yakima, Wash., 1923*

Test No.	Material	Number of tests	Number of wormholes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per wormhole
1	Lead arsenate, 1–50	4	38	19.0	47	23.5	42.5	1.24
2	Lead arsenate, 2–50	4	36	18.0	35	17.5	35.5	.97
3	Control (unsprayed)	4	132	66.0	7	3.5	69.5	.05

TABLE 4.—*Comparison of the effectiveness of different strengths of powdered lead arsenate when used with and without casein spreader against the larva of the codling moth, Yakima, Wash., 1924*

Test No.	Material	Number of tests	Number of wormholes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per wormhole
1	Lead arsenate, ½–50; no spreader	4	50	25.0	30	15.0	40.0	0.60
2	Lead arsenate, ¾–50; no spreader	4	36	18.0	36	18.0	36.0	1.00
3	Lead arsenate, 1–50; no spreader	4	37	18.5	40	20.0	38.5	1.08
4	Lead arsenate, 2–50; no spreader	4	30	15.0	41	20.5	35.5	1.37
5	Lead arsenate, 3–50; no spreader	4	19	9.5	46	23.0	32.5	2.42
6	Lead arsenate, ½–50; casein spreader, ¼–50	3	29	19.3	25	16.7	36.0	.86
7	Lead arsenate, ¾–50; casein spreader, ¼–50	3	17	11.3	34	22.7	34.0	2.00
8	Lead arsenate, 1–50; casein spreader, ¼–50	3	9	6.0	37	24.7	30.7	4.11
9	Lead arsenate, 2–50; casein spreader, ¼–50	3	4	2.7	28	18.7	21.4	7.00
10	Lead arsenate, 3–50; casein spreader, ¼–50	3	3	2.0	42	28.0	30.0	14.00
11	Control (unsprayed)	4	110	55.0	4	2.0	57.0	.04

LIGHT AND HEAVY APPLICATIONS OF LEAD ARSENATE

The general opinion has been that where no spreader is used, it is more desirable to cover the fruit evenly with fine drops of spray than to "overspray" it and leave coarse drops on the surface with apparently unprotected areas between them. In Table 5 the results of

tests of this nature are recorded. In tests 1 and 4 (light applications) the fruit was thoroughly covered with small drops of spray, whereas in tests 2 and 5 (heavy applications) it was very much oversprayed and much of the spray dripped off. Most of the spray left on the fruit collected in large drops. The results in 1923 and 1924 are rather in favor of the heavy application, although the difference is not great, and, curiously, the number of stings is reduced more than the number of worms by the heavy application. From the standpoint of the ratio of stings to wormholes, the light application was better than the heavy application.

TABLE 5.—*Comparison of the effectiveness of light and heavy applications of powdered lead arsenate when used against the larva of the codling moth, Yakima, Wash., 1923 and 1924.*

Test No.	Material	Number of tests	Number of wormholes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per wormhole
	1923							
1	Lead arsenate, 1-50; light application	4	37	18.5	54	27.0	45.5	1.46
2	Lead arsenate, 1-50; heavy application	4	36	18.0	36	18.0	36.0	1.00
3	Control (unsprayed)	4	132	66.0	10	5.0	71.0	.08
	1924							
4	Lead arsenate, 1-50; light application	3	16	10.7	44	29.3	40.0	2.75
5	Lead arsenate, 1-50; heavy application	3	13	8.7	29	19.3	28.0	2.23
6	Control (unsprayed)	3	71	47.3	11	7.3	54.6	.15

PASTE VERSUS POWDERED LEAD ARSENATE

The powdered lead arsenate has almost entirely displaced the paste form, but there has been some question as to whether or not it is as effective. Scott and Siegler (*16*) obtained practically identical results in laboratory tests against the fall webworm with paste and powdered lead arsenate used at equivalent strengths, and also in orchard tests against the codling moth. Siegler and Plank (*18*) used paste lead arsenate in 1915 and powdered lead arsenate in 1916, 1917, and 1918, in orchard tests against the codling moth. It is difficult to compare tests made in different years, but the results do not indicate any advantage of one form of lead arsenate over the other. De Sellem (*4*) obtained somewhat better results with powdered lead arsenate at 1 pound to 50 gallons than with paste lead arsenate at 2 pounds to 50 gallons.

The paste lead arsenate was tested by the writer in 1924. Since the manufacturer of the particular brand employed recommended its use at 7 pounds to 200 gallons instead of the customary 8 pounds, it was used at that strength and compared with the powdered form at an equivalent strength—that is, at seven-eighths pound to 50 gallons—and also at the usual 1 pound to 50 gallons. This experiment, recorded in Table 6, does not indicate that the paste form is superior to the powdered, although the results with the paste were slightly better than those with an equivalent amount of powder. The paste lead arsenate used in this test contained 15.21 per cent of As_2O_5, and the powdered lead arsenate was guaranteed to contain 31 per cent of As_2O_5.

TABLE 6.—*Comparison of effectiveness of paste and powdered lead arsenate when used against the larva of the codling moth, Yakima, Wash., 1924*

Test No.	Material	Number of tests	Number of wormholes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per wormhole
1	Powdered lead arsenate, 1-50; no spreader	5	48	19.2	57	22.8	42.0	1.19
2	Powdered lead arsenate, ⅞-50; no spreader	5	47	18.8	74	29.6	48.4	1.57
3	Paste lead arsenate, 1¾-50; no spreader	5	40	16.0	68	27.2	43.2	1.70
4	Powdered lead arsenate, 1-50; casein spreader, ¼-50	3	13	8.7	26	17.3	26.0	2.00
5	Powdered lead arsenate, ⅞-50; casein spreader, ¼-50	3	20	13.3	40	26.7	40.0	2.00
6	Paste lead arsenate, 1¾-50; casein spreader, ¼-50	3	16	10.7	30	20.0	30.7	1.88
7	Control (unsprayed)	5	140	56.0	14	5.6	61.6	.10

LEAD ARSENATE IN COMBINATION WITH LIME-SULPHUR AND OIL EMULSIONS

It is often desired to combine the summer strength lime-sulphur used for mildew and other fungous diseases with the lead arsenate, and the increasing use of summer oil sprays suggested the possibility of combining these with the lead arsenate. Accordingly, these combinations have been tested, acid lead arsenate being used.

According to Robinson (*14*), adding lime-sulphur to acid lead arsenate increases the amount of soluble arsenic and reduces the amount of arsenic oxide (As_2O_5). A large amount of lead sulphide (PbS) is also produced. Gray (*6*) questions the advisability of mixing lime-sulphur with acid lead arsenate. Sanders and Brittain (*15*) report that in field tests with various insect larvae the addition of lime-sulphur to the lead arsenate reduced its effectiveness, and Scott and Siegler (*16*) found that the killing action of the arsenical was slower when lime-sulphur was added than when the arsenical was used alone. Cook and McIndoo (*3*) obtained somewhat better results when the lead arsenate was used alone than when lime-sulphur was used with it. The writer, in various orchard tests against the codling moth at Yakima and Wenatchee, Wash., the results of which have not been published, has obtained poorer results when lime-sulphur was added than when the lead arsenate was used alone.

In the laboratory tests reported in Tables 7 and 8, a very marked increase in the number of wormholes resulted from the addition of lime-sulphur, the number being nearly twice as great in 1923, and more than twice as great in 1924. The number of stings was correspondingly decreased, and there was little difference in the total number of blemishes.

The addition of lime to this combination spray has been shown by Robinson (*14*) to prevent any reaction between the lime-sulphur and the lead arsenate, and Regan (*13, p. 47*) reports that the addition of casein spreader (a mixture of casein and lime) prevents this reaction. In test 3 (Tables 7 and 8) the casein spreader was used, it being placed in the water before the other ingredients were added. No reaction was noticeable, and the results are much better than those in test 2 where the combination spray was used without spreader, and even better than in test 1 where lead arsenate was used alone. This latter difference may be due entirely to the better coating obtained with the spreader.

In 1923, a lubricating-oil-emulsion made with soap according to W. W. Yothers's formula (*23, p. 19*) was mixed with the lead arsenate. Four tests were made, with rather variable results, the average, as shown in Table 7, test 4, being somewhat poorer than with the lead arsenate alone. Gray (*6*) states that soap and lead arsenate are incompatible, and Pinckney (*12*) found that soap increased the amount of soluble arsenic, but he does not mention whether it affects the toxicity of the arsenical. The variation in the results may have been due to a reaction between the lead arsenate and the soap, and, if so, it may be inadvisable to mix these two materials.

In 1924, the casein lubricating-oil emulsion prepared according to formula No. 3 of Burroughs and Grube (*2*) was used with lead arsenate in test 4 (Table 8). The results were superior to those obtained from the lead arsenate alone.

TABLE 7.—*Comparison of the effectiveness of powdered lead arsenate alone and in combination with lime-sulphur and lubricating-oil emulsion, when used against the larva of the codling moth, Yakima, Wash., 1923*

Test No.	Material	Number of tests	Number of wormholes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per wormhole
1	Lead arsenate, 1-50; no spreader	4	43	21.5	46	23.0	44.5	1.07
2	Lead arsenate, 1-50; commercial 30° lime-sulphur, 1-50	4	74	37.0	23	11.5	48.5	.31
3	Lead arsenate, 1-50; commercial 30° lime-sulphur, 1-50; casein spreader, ¼-50	4	47	23.5	30	15.0	38.5	.64
4	Lead arsenate, 1-50; soap-oil emulsion, ½-50	4	55	27.5	24	12.0	39.5	.44
5	Control (unsprayed)	4	138	69.0	8	4.0	73.0	.06

TABLE 8.—*Comparison of the effectiveness of powdered lead arsenate alone and in combination with lime-sulphur and lubricating-oil emulsion, when used against the larva of the codling moth, Yakima, Wash., 1924*

Test No.	Material	Number of tests	Number of wormholes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per wormhole
1	Lead arsenate, 1-50; no spreader	4	31	15.5	54	27.0	42.5	1.74
2	Lead arsenate, 1-50; commercial 30° lime-sulphur, 1-50	3	50	33.3	23	15.3	48.6	.46
3	Lead arsenate, 1-50; commercial 30° lime-sulphur, 1-50; casein spreader, ¼-50	3	12	8.0	23	15.3	23.3	1.92
4	Lead arsenate, 1-50; casein-oil emulsion, ½-50	4	21	10.5	43	21.5	32.0	2.05
5	Control (unsprayed)	4	84	42.0	16	8.0	50.0	.19

VARIOUS ARSENICALS

Arsenicals other than lead arsenate have been used extensively in laboratory and field tests, chief among them being calcium arsenate and zinc arsenite. Paris green was formerly used, but owing to its poor adhesive qualities and its tendency to burn the foliage, it is now seldom used on fruit trees.

A review of some of the reports of tests of calcium arsenate shows a considerable diversity of results. Wilson (*22*) noted no difference in

the effect of calcium arsenate and lead arsenate on tent caterpillars. Lovett and Robinson (*7*) obtained equal results with the two materials used against leaf-feeding insects, as did Scott and Siegler (*16*), but the latter found that the calcium arsenate killed somewhat more slowly. Scott and Siegler's results with calcium arsenate in orchard tests against the codling moth indicated it to be inferior to the lead arsenate in two tests and about equal to it in a third test. W. M. Scott (*17*) reported that both the paste and the powdered calcium arsenate were equal to lead arsenate in orchard tests, and De Sellem (*4*) found the same thing to be true under favorable conditions. Lovett (*8*) obtained better results with calcium arsenate than with lead arsenate in the orchard, but Siegler and Plank (*18*) and Newcomer (*11*) obtained inferior results under wormy conditions. Regan (*13, p. 48*), testing calcium arsenate against the fruit-tree leaf roller, obtained poor results as compared with those obtained with lead arsenate.

Cook and McIndoo (*3*) made extensive experiments with various arsenicals against leaf-feeding insects. They obtained results comparable to those with lead arsenate with only one of six samples of commercial calcium arsenate tested. Paris green gave uniformly better results than the lead arsenate, and zinc arsenite gave somewhat poorer results. Melander (*9, p. 23-24*), reported very good results with zinc arsenite, under favorable conditions, but they were not equal to those obtained with lead arsenate.

Tables 9 and 10 give the results obtained by the writer with various arsenicals at Yakima, Wash., in 1923 and 1924. The powdered zinc arsenite, which was guaranteed to contain 30.5 per cent of arsenic expressed as metallic arsenic, was decidedly inferior to the lead arsenate when used at 1 pound to 50 gallons, and was still inferior when used at 2 pounds to 50 gallons.

Paris green, guaranteed to contain more than 50 per cent arsenious trioxide, was used in 1923, at one-half pound to 50 gallons, with an equal amount of lime, and also with casein spreader, and it produced slightly better results than lead arsenate. In 1924 it was used at two-fifths pound to 50 gallons, with an equal amount of lime, and also with casein spreader, and the results were not quite as good as with lead arsenate.

Powdered calcium arsenate, guaranteed to contain not less than 40 per cent arsenic pentoxide, was used in 1923 at three-fourths pound to 50 gallons, a strength equivalent to 1 pound of powdered lead arsenate to 50 gallons. The results with the calcium arsenate were decidedly inferior to those with lead arsenate, more than twice as many larvae entering the fruit where no spreader was used, and nearly three times as many where the casein spreader was added. In 1924, a paste calcium arsenate was tested, which analyzed 15.19 per cent arsenic oxide. This was used at 2 pounds to 50 gallons, and the results were practically identical with those obtained from the use of powdered lead arsenate at 1 pound to 50 gallons. When casein spreader was added, however, the results with calcium arsenate compared with lead arsenate were not nearly so good, indicating a possible reaction between the lime in the casein spreader and the calcium arsenate.

TABLE 9.—*Comparison of the effectiveness of various arsenicals when used with and without casein spreader against the larva of the codling moth, Yakima, Wash., 1923*

Test No.	Material	Number of tests	Number of wormholes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per wormhole
1	Lead arsenate (powdered), 1–50	4	47	23.5	47	23.5	47.0	1.00
2	Zinc arsenite (powdered), 1–50	4	83	41.5	39	19.5	61.0	.47
3	Paris green, ½–50; lime, ½–50	3	27	18.0	29	19.3	37.3	1.07
4	Calcium arsenate (powdered), ¾–50	4	97	48.5	30	15.0	63.5	.31
5	Lead arsenate (powdered), 1–50; casein spreader, ¼–50	3	15	10.0	33	22.0	32.0	2.20
6	Zinc arsenite (powdered), 1–50; casein spreader, ¼–50	3	39	26.0	32	21.3	47.3	.82
7	Paris green, ½–50; casein spreader, ¼–50	3	13	8.7	29	19.3	28.0	2.23
8	Calcium arsenate (powdered), ¾–50; casein spreader, ¼–50	3	44	29.3	44	29.3	58.6	1.00
9	Control (unsprayed)	4	128	64.0	10	5.0	69.0	.08

TABLE 10.—*Comparison of the effectiveness of various arsenicals when used with and without casein spreader against the larva of the codling moth, Yakima, Wash., 1924*

Test No.	Material	Number of tests	Number of wormholes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per wormhole
1	Lead arsenate (powdered), 1–50	3	35	23.3	42	28.0	51.3	1.20
2	Zinc arsenite (powdered), 1–50	3	59	39.3	14	9.3	48.6	.24
3	Zinc arsenite (powdered), 2–50	3	42	28.0	28	18.7	46.7	.67
4	Paris green, ⅔–50; lime, ⅔–50	3	39	26.0	33	22.0	48.0	.85
5	Calcium arsenate (paste), 2–50	3	35	23.3	43	28.7	52.0	1.23
6	Lead arsenate (powdered), 1–50; casein spreader, ¼–50	3	11	7.3	47	31.3	38.3	4.27
7	Zinc arsenite (powdered), 1–50; casein spreader, ¼–50	3	28	18.7	32	21.3	40.0	1.14
8	Zinc arsenite (powdered), 2–50; casein spreader, ¼–50	3	16	10.7	37	24.7	35.4	2.31
9	Paris green, ⅔–50; casein spreader, ¼–50	3	18	12.0	35	23.3	35.3	1.94
10	Calcium arsenate (paste), 2–50; casein spreader, ¼–50	3	30	20.0	22	14.7	34.7	.73
11	Control (unsprayed)	3	84	56.0	8	5.3	61.3	.10

CALCIUM ARSENATE

As already indicated, the results obtained by various investigators with calcium arsenate have been rather variable. Apparently this material may be depended on to give good control where the results are secured within a few days. This is particularly true of leaf-feeding insects, which eat comparatively large quantities of food, and which may not ingest a lethal dose of poison for several days. In the case of the codling moth, where the poison is expected to be effective over a period ranging from 10 days to a month, and where the insect does only a limited amount of feeding on the poisoned surface and must therefore take in a lethal dose within a short time, the control has been quite variable. This has evidently been due partially to a varying degree of infestation, but the work of Cook and McIndoo (*3*) would indicate also a varying degree of effectiveness in different brands of calcium arsenate. In order to test this point with the codling moth, four brands of powdered calcium arsenate

were used in 1923, and one brand of paste calcium arsenate in 1924. Comparisons were made with powdered lead arsenate used at an equivalent strength.

The powdered calcium arsenates used in 1923 contained the following percentages of arsenic pentoxide: Brand A, not less than 40 per cent by guarantee; brand B, 39.93 per cent, by analysis; brand C, 39.21 per cent, by analysis; and brand D, not less than 40 per cent, by guarantee. Table 11 shows that all of these samples were inferior to the lead arsenate in controlling the codling moth. Brands B and D were better than the other two, and these were fluffier and apparently more finely ground than brands A and C.

In 1924, a paste calcium arsenate was tested (brand E) which contained, by analysis, 15.19 per cent of arsenic pentoxide. The results obtained with this brand at 2 pounds to 50 gallons were almost identical with those obtained from an equivalent amount of powdered lead arsenate. Used at 1 pound to 50 gallons, the control obtained was somewhat poorer, but still fair. The addition of casein spreader to this calcium arsenate evidently interfered with its action, as the results were much inferior to those with lead arsenate and casein spreader. These results are recorded in Table 11.

Apparently there is a considerable difference in the effectiveness of different brands of calcium arsenate, and this difference is not due to a variable amount of arsenic present. It is possibly a physical difference, some kinds adhering better than others. The results with brand E indicate that a calcium arsenate can be made equal to lead arsenate in controlling the codling moth.

TABLE 11.—*Comparison of the effectiveness of various brands of calcium arsenate with lead arsenate when used against the larva of the codling moth, Yakima, Wash., 1923 and 1924*

Test No.	Material	Number of tests	Number of wormholes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per wormhole
	1923							
1	Lead arsenate (powdered), 1–50	3	21	14.0	47	31.3	45.3	2.24
2	Calcium arsenate (brand A), ¾–50	3	51	34.0	46	30.7	64.7	.90
3	Calcium arsenate (brand B), ¾–50	3	45	30.0	43	28.7	58.7	.96
4	Calcium arsenate (brand C), ¾–50	2	42	42.0	25	25.0	67.0	.60
5	Calcium arsenate (brand D), ¾–50	2	29	29.0	38	38.0	67.0	1.31
6	Control (unsprayed)	3	119	79.3	5	3.3	82.6	.04
	1924							
7	Lead arsenate (powdered), 1–50	3	35	23.3	42	28.0	51.3	1.20
8	Calcium arsenate (brand E), 1–50	3	46	30.7	33	22.0	52.7	.72
9	Calcium arsenate (brand E), 2–50	3	35	23.3	43	28.7	52.0	1.23
10	Lead arsenate (powdered), 1–50; casein spreader, ¼–50	3	11	7.3	47	31.3	38.6	4.27
11	Calcium arsenate (brand E), 1–50; casein spreader, ¼–50	3	67	44.7	21	14.0	58.7	.31
12	Calcium arsenate (brand E), 2–50; casein spreader, ¼–50	3	30	20.0	22	14.7	34.7	.73
13	Control (unsprayed)	3	84	56.0	8	5.3	61.3	.10

SUMMARY

The difficulty of obtaining uniform working conditions when making orchard tests for the control of the codling moth has suggested the desirability of more exact laboratory methods. The method of spraying individual apples and placing a given number of larvae on them has been employed in the investigation reported in this paper. It is not possible to investigate all phases of codling-moth control by this method, but it is believed that certain comparisons may be made more accurately in this way than by field tests. It is to be understood that the tests here reported show the effect of the various sprays only when freshly applied.

Almost without exception, the addition of casein spreader to lead arsenate or to other arsenicals or combination sprays materially improved the control obtained. The smallest amount of casein spreader used, one-eighth of a pound to 50 gallons, gave better control when used with lead arsenate than any larger amount tested.

Increasing the strength of the lead arsenate reduced the number of wormholes and generally increased the number of stings, the total number of blemishes being reduced very little.

A heavy application of lead arsenate gave somewhat better results than a light application.

No appreciable difference was observed in the effectiveness of equivalent amounts of paste and powdered lead arsenate.

The addition of lime-sulphur to acid lead arsenate materially reduced its efficiency, but this reduction was overcome by the use of casein spreader with the combination spray. The lime in the spreader apparently prevents or retards the usual reaction between lime-sulphur and acid lead arsenate.

The addition of a lubricating-oil emulsion containing soap as an emulsifier produced variable results, the average being poorer than those obtained with lead arsenate alone. A lubricating-oil emulsion containing casein as an emulsifier did not have this effect, the control being somewhat improved over that obtained from lead arsenate alone.

Zinc arsenite was not as effective as lead arsenate. Paris green, with lime or with casein spreader, gave slightly better results in 1923, but in 1924 the control did not quite equal that obtained from lead arsenate. Powdered calcium arsenate gave very poor control, although four brands were tested. A paste calcium arsenate equalled an equivalent amount of powdered lead arsenate.

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LIFE HISTORY OF THE HICKORY SPIRAL BORER, AGRILUS ARCUATUS SAY[1]

By FRED E. BROOKS

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INTRODUCTION

In the eastern part of the United States there are several species of beetles the larvae and adults of which kill hickory and pecan branches and young trees by girdling or otherwise severing the wood in a vital place. In all cases it seems that the purpose of the insect in doing the injury is to provide itself with dead or dying wood in which to undergo part or all of its development. This paper deals with one of these beetles, which may be called the hickory spiral borer because of the peculiar winding burrow which is the chief injury that it does to trees. In many instances medium-sized branches of bearing trees are killed by this borer, which reduces the crop of nuts, but the principal loss is in the injury to small trees. Young hickory trees in forests, in orchards, and on lawns, and especially in nurseries where seedlings and grafted trees are produced for planting, are liable to attack.

This beetle belongs to the family Buprestidae, the members of which are often called flat-headed borers or metallic wood borers. The term "flat-headed" refers to the broad, flattened front segments of the grubs of some species; and the term "metallic" to the lustrous, metallic colors often displayed by the beetles. The larvae of the many species of Agrilus, to which genus the hickory spiral borer belongs, mine in the twigs, stems, roots, and beneath the bark of trunks and branches of numerous kinds of trees and smaller plants. The group includes such familiar pests as the raspberry cane borer (*Agrilus ruficollis* Fab.), the two-lined chestnut borer (*A. bilineatus* Web.), the bronze birch borer (*A. anxius* Gory), and the Pacific oak twig girdler (*A. angelicus* Horn).

IDENTITY AND DISTRIBUTION

According to W. S. Fisher of the Bureau of Entomology of the United States Department of Agriculture, the forms of Agrilus related to *Agrilus arcuatus* are closely allied but not sufficiently well characterized to identify them with certainty. The form which feeds on hickory and pecan is believed to represent only one species, and it is treated in this paper under the name *arcuatus*. Certain varieties of *arcuatus* have been recognized by Le Conte, but they resemble one another so closely that it seems likely that misidentifications have been made. For this reason it does not seem desirable to summarize the literature which has appeared under the name *arcuatus* or its varieties.

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This confusion of names makes it uncertain as to how widely the species here called the hickory spiral borer is distributed, and whether other trees than hickory and pecan are sometimes attacked. There

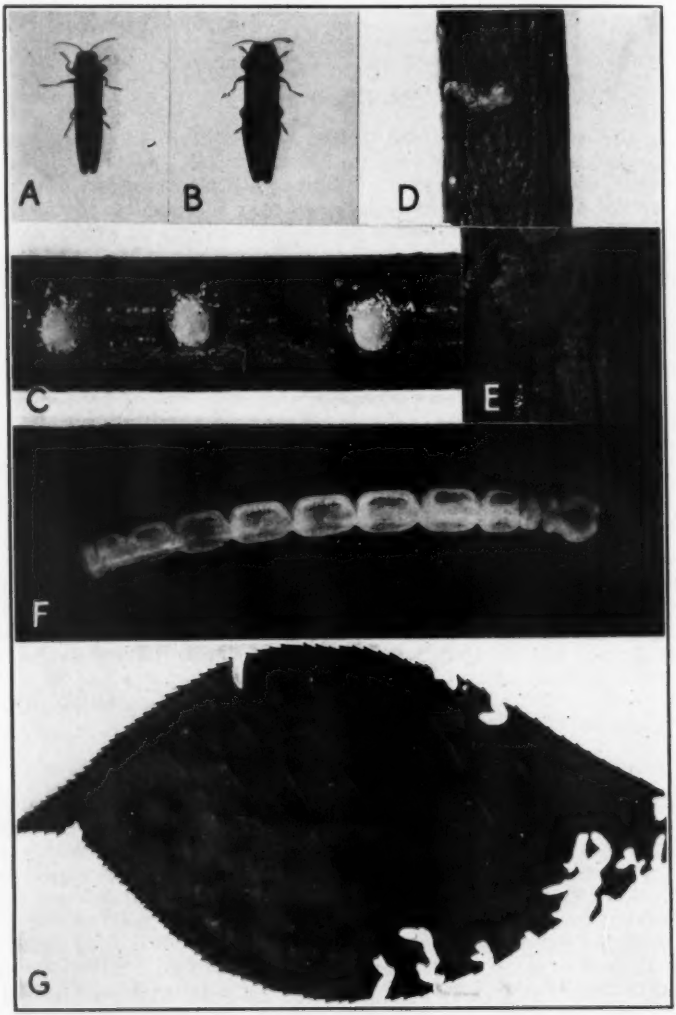

FIG. 1.—Hickory spiral borer: A, male beetle, × 2; B, female bettle, × 2; C, eggs, × 5; D, larva issuing from egg, × 5 (the eggshell has been removed to the left from over the larva in order to disclose the hatching larva); E, exit hole of beetle, × 2; F, larva, × 4; G, hickory leaf showing feeding marks of the beetle, natural size

seem to be valid records of its occurrence in New York, Pennsylvania, and Virginia. The present writer has collected the insect or has seen specimens of its work at Morgantown, Pickens, French Creek,

Buckhannon, Moorefield, Great Cacapon, and Clarksburg, W. Va.; and at Pulaski, Richmond, Petersburg, and Round Hill, Va. Severed hickory branches, apparently the work of this insect, were found by the writer in the vicinity of Storrs, Conn.

NATURE AND EXTENT OF INJURY

The hickory spiral borer hatches from an egg laid on the bark of a twig (fig. 1, C), and lives as a larva in the tree from 22 to 23 months, the period including two entire winters. The larva (fig. 1, F) spends most of the summer burrowing just under the bark, occasionally going deeper into the wood and sometimes following the pith for short distances. Its general course in the wood is downward. Each winter it makes a winding, concentric cut from the inner bark to the heart of the branch or stem, the coils of the thin burrow joining and completely severing the wood except for the bark and sometimes a slender fiber of wood at the heart (fig. 2, C and D). These spiral burrows are in process of making in late autumn, in warm periods in winter, and in the early spring. The first of the two spiral burrows which each insect makes severs a small twig or terminal, and the second severs a larger part of the wood. The portion above the spiral wound dies in the spring before the foliage appears, the injury becoming apparent as the rest of the tree puts forth leaves (fig. 3). Trees and branches from the diameter of a lead pencil to nearly 2 inches in diameter are severed.

The borer is native to the eastern forests where hickory trees abound, and in several instances it has been observed injuring hickory and pecan trees in nurseries and nut-tree orchards in the eastern part of the country. In a nursery at Petersburg, Va., in 1923 and 1924 it did serious damage to seedling and grafted hickories and pecans. In one small block of 5-year-old hickory seedlings in this nursery an hour's search revealed 54 trees with their main stalks recently severed by 2-year-old larvae, and an equal number of twigs and terminals similarly injured by 1-year-old larvae. The trees which the older larvae had severed were set back several years in growth, many of them being practically ruined. Oftentimes such young trees with trunks thus severed will put forth another shoot which will in turn be cut off within a few years. In many instances in localities where the insect is abundant, hickory bushes which have become stunted by frequent pruning will show from 5 to 10 fresh and old wounds. The greatest injury to plantations has been observed near woods in which hickory grows. However, trees in open fields, as well as in the shadow of forest, are liable to attack.

LIFE HISTORY

THE EGG

The eggs (fig. 1, C) are flat, disklike, somewhat irregular in shape and size, and are glued firmly to the smooth bark of twigs. They resemble quite closely the shield of a small scale insect. When first laid the egg is smooth and pale yellowish green, but before hatching it becomes slightly wrinkled and almost black. The diameter ranges from 0.8 mm. to 1.1 mm. When laid they are partially concealed with a light covering of grains of excrement (fig. 1, C), but the covering soon drops away, and as the egg deepens in color, a

transparent film appears around it. About a dozen eggs collected at different times hatched in 26 and 27 days. When confined in cages the beetles often laid several eggs in a more or less compact group, but in the field not more than one egg was found in a place.

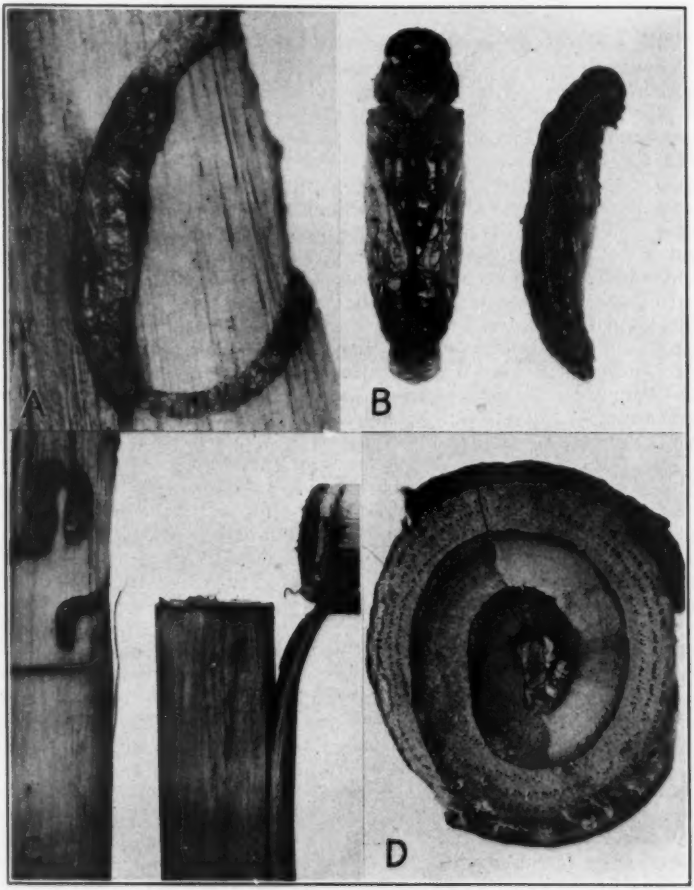

FIG. 2.—Hickory spiral borer: A, pupa in natural position, × 5; B, pupae, × 5; C, hickory stalks showing the burrows; D, hickory branch at point of severance showing spiral girdle of larva, × 2

THE LARVA

The larva (fig. 1, F) is a slender, flat, legless grub, full-grown specimens being from 15 mm. to 20 mm. long and about 2 mm. wide. The color is yellowish white, like that of the wood in which it feeds, except that the small mouth parts and the tail forceps are dark brown or black. It is bare of hairs, save for a small patch of short, whitish bristles at the base of the tail forceps. It is sluggish in movement at all times.

The larva leaves the egg in midsummer, eating its way through the bottom of the egg and directly into the twig. In the twig it makes elongate, threadlike burrows next to the bark and through the wood. Late in autumn it begins a spiral burrow which even-

FIG. 3.—Top of a young hickory tree killed by larvae of the hickory spiral borer

tually severs the wood and kills the terminal above. The spiral burrow may be completed in the autumn, or in the following spring. In either case the larva spends the winter in and about the spiral wound. In all its mining the borer packs the gallery behind with fine wood dust.

With the coming of warm weather the borer works below the severed point, first moving downward for an inch or two in the pith and then tunneling out to the bark. Through the summer it proceeds downward a foot or more, feeding with its back to the inner bark and leaving a shallow but relatively wide burrow packed with wood-colored dust. Late in the season it changes its course abruptly and, with its side to the bark, cuts a thin, symmetrical ring around the branch (fig. 2, C and D). When the first circuit is completed it directs its boring operations spirally inward in the same plane, encircling the stem again, and it continues the spiral burrowing until the heart is reached, when the terminal above will break off with the application of slight force. When this second spiral boring is completed the borer turns upward and eats its way from the heart directly to the bark at a point about half an inch or less above the outer tunnel of the spiral boring (fig. 2, C). By the time it has arrived at the bark, spring has returned, and the larva is then nearly full-grown. It then proceeds to make a crooked burrow a few inches in length just beneath the bark (fig. 2, C) after which it forms a crescent-shaped pupal chamber, the ends of the chamber extending to the bark and the bottom curving toward the heart of the wood (fig. 2, A). Both ends of the chamber are filled with dust, and the larva occupies the somewhat enlarged space between. The larva soon shrinks to about half its former length, and in about a week it changes to the pupal stage.

THE PUPA

The pupa (fig. 2, A and B) when first formed is a delicate, white, somewhat curved object, the back being flattish and the underparts bearing outlines of the antennae, wings, and legs. It ranges from 9 to 12 mm. in length, and from 2 to 3 mm. in width. Within a week the eyes begin to darken, and soon thereafter the head, thorax, and other parts of the body change to purplish black, the shades of color deepening until the beetle stage is reached. The pupae are present in May and June, the pupal stage of development lasting about 20 days. The pupa rests in an almost vertical position in a pupal chamber which curves inward and downward from the bark, usually to the heart of the branch. The middle section of the chamber has been enlarged somewhat by the larva for the more comfortable accommodation of its body (fig. 2, A).

THE ADULT

The adults (fig. 1, A and B) are dark and slender, the males averaging considerably smaller than the females. The head and thorax of the male are greenish bronze, the elytra are purplish black, and the underparts are brassy; the female is bronze in color effect throughout. The average length of the male is about 8 mm., and that of the female about 10 mm. The beetles appear from May to July, are active only on warm days, and live for about two months. Most of the eggs are deposited in July and the first half of August.

The beetle makes its escape from the pupal chamber through a small D-shaped hole which it gnaws through the bark (fig. 1, E). This hole is usually from 1 to 3 inches above the spiral wound which kills the terminal. The beetles appear from May to July, emergence of individuals in a given locality occurring over a period of at least 25 days, and the season of emergence varying in different localities.

In the summer of 1924 a number of beetles were reared from hickory wood collected at Petersburg, Va., and French Creek, W. Va. Petersburg is approximately 115 miles south of French Creek, and is perhaps 1,400 feet nearer sea level than French Creek. As would be expected, the beetles from the more southerly locality, although belonging to the same generation, transformed and issued first. The beetles reared from the material collected at Petersburg numbered 19, and they issued from May 30 to June 13, the maximum emergence being from May 30 to June 10. The number reared from the material collected at French Creek was 111, and emergence continued from June 23 to July 17, the maximum emergence being from June 24 to June 30. The beetles from Petersburg appeared, on an average, from 20 to 30 days ahead of those from French Creek.

The males appear a few days before the females, and they also die first. In a lot of 10 pairs confined and fed in glass jars, all the females survived the males. Practically all the beetles left the wood in the morning and began to feed soon after they issued, making elongate notches and slits in the edges of the leaves (fig. 1, G). Mating took place within from 12 to 24 hours after emergence, and the first eggs were, as a rule, deposited from 10 days to two weeks later. A female in the act of egg laying was observed to first spend several minutes moving over the bark with the tip of her abdomen pressed against the surface as though searching for a suitable place to deposit her eggs. When such a place was found she devoted about a minute to scraping the spot with her anal tip to smooth or moisten the surface. She then laid an egg and immediately began to move the tip of her body rapidly across and in circles over the egg and the bark immediately surrounding it. This performance lasted 90 seconds, and when it was finished the egg was thinly covered with a transparent liquid, present in slight amount, which glued the egg to the bark.

On July 1, 1924, ten pairs of beetles which had recently issued in rearing jars and which had not begun to oviposit, were found in copulation, and they were confined by pairs in glass jars in an open insectary. They were provided daily with fresh hickory leaves for food and fresh hickory twigs on which to oviposit. A record was kept of the number of eggs laid by each female and the time of death of all individuals.

The 10 females laid 226 eggs, an average of 22.6 each. The greatest number laid by one female was 55, and the smallest number was 2. Eggs were laid during a period of 51 days. Two females of the lot issued from the wood on June 23, first mated on June 24, and lived until August 29, a period of 67 days. The greatest number of eggs laid in one day by the 10 females was 15; this was on July 27. The period of maximum egg production was from July 12 to August 8. The females, on an average, lived about three weeks longer than the males.

NATURAL ENEMIES

Several species of hymenopterous parasites were found killing the larvae of the borer, although a relatively small percentage of the insects were parasitized. These Hymenoptera included *Labena apicalis* Cress., determined by R. A. Cushman; and *Monogonogastra agrili* (Ashm.) and *Zatropus* sp. near *nigroaeneus* (Ashm.), determined by S. A. Rohwer.

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METHODS OF CONTROL

It seems probable that the hickory spiral borer may be held in check in nurseries and other plantations of small hickory and pecan trees by dusting or spraying with arsenicals. Beetles kept in cages ate rather freely of the leaves with which they were provided, and they responded to the poison when it was given to them on sprayed foliage. In one instance 20 beetles, equally divided as to sex, were placed in a roomy cage over a live hickory branch that had been sprayed with lead arsenate at a strength of 2 pounds of powdered arsenate to 50 gallons of water. Two days later 9 of the beetles were dead, in 3 days 18 were dead, and the other 2 died in 4 and 5 days, respectively. In a near-by cage set up as a check 50 beetles were confined over an unsprayed branch, and not one of these died during the period of the test. Arsenicals for the control of the borer should be applied from the first to the last of June, according to the locality.

Infested young trees in nurseries and orchards should be pruned of the killed branches and terminals as soon as the leaves develop in the spring. By pruning thus early the insects will be collected with the prunings and may be destroyed by burning. Special care should be taken to cut off the small dead twigs that have been severed by the first-winter larvae. Such twigs should be clipped a few inches below the dead part in order to make sure of getting the borer, which starts to move down the stem at the beginning of warm weather. Twigs containing the 1-year-old larvae need not necessarily be burned, as the borers within them will die when the twigs are cut off. By so disposing of the young borers the more serious damage which they would inflict later may be avoided.

THE PINE BUTTERFLY, NEOPHASIA MENAPIA FELDER[1]

By JAMES C. EVENDEN

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INTRODUCTION

Thousands of acres of yellow pine along the Little Salmon and Payette Rivers, Idaho, were severely defoliated by the pine butterfly (*Neophasia menapia* Felder) in 1922 and 1923. Much has been published concerning this insect, and very accurate descriptions of the various stages of its development have been given (*7*, *2*, *4*),[3] but no data are available relative to its seasonal history, and conflicting ideas have been advanced as to the number of generations per season (*1*, *6*). Therefore it seems desirable to publish the results of the last two years' field and laboratory study of the insect in Idaho, including brief descriptions of the egg, larva, and imago, together with its distribution and its hosts.

DISTRIBUTION AND HOSTS

Epidemics of the pine butterfly have been recorded from various sections of the Northwest (*5*, *7*). The insect has also been collected in California, and it is found as far east as the eastern or front range of the Rocky Mountains. Briefly, it may be considered as present in all of the pine forests in the western part of the United States and Canada.

Practically all species of pine throughout the range of the insect are attacked to a greater or less extent, but western yellow pine (*Pinus ponderosa* Laws.) is the preferred host (fig. 1).

Fletcher (*3*) records the pine butterfly as severely injuring Douglas fir (*Pseudotsuga taxifolia* (Poir) Britt.) in the coast regions of British Columbia. The writer has no record of injury to trees other than to pine in Idaho.

DESCRIPTION

ADULT

Stretch (*7*) has very accurately described both the male and female. Nevertheless a brief description intended for field determinations may not be out of place in this paper. The pine butterfly very closely resembles the common cabbage Pieris. It has a wing expanse of about 43 mm.; antennae black; head and body black above and white beneath, covered with hairs (fig. 2, E).

MALE.—The scales of the fore wings of the male are pure white, except for the black markings on the tips and a streak along the costal vein. On the under side the same general markings occur.

The hind wings are white, except along the tips, which are lightly touched with black. On the under side the markings are heavier, and the black veins show through on the upper side.

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[1] Received for publication Jan. 26, 1926; issued August, 1926.

[2] The writer wishes to acknowledge the assistance given by H. J. Rust, senior scientific aid, Bureau of Entomology, in conducting the rearing experiments in connection with the laboratory studies.

[3] Reference is made by number (italic) to "Literature cited," p. 344.

FEMALE.—The scales of the fore wings of the female are of a light yellowish color. The black markings are like those of the male, with a black apical margin in addition. On the under side the markings are the same.

The hind wings have the same yellowish tint as the fore wings, but they differ from those of the male in having heavier black lines along the tips. More black appears on the under side, all of the veins being marked with broad lines. On many specimens, but not all, there are bright orange-red spots along the apical margin.

EGG

The eggs (fig. 2, A), 1½ mm. in length, are laid along the pine needle, in rows of 5 to 20, at an angle of 45° pointing toward the

FIG. 1.—Stand of western yellow pine defoliated by the pine butterfly

end of the needle, and are firmly cemented together. Fletcher (*3*) has described them as the "most beautiful objects, resembling minute emerald-green Florence flasks, vertically lined with delicate lines and with a beaded rim of porcelain-white knobs."

LARVA

Although the larval stages have been described fully by Edwards (*2*), a brief description of the young and mature larvae is included here for the convenience of the reader in determining the insect in the field. The larva is 2 mm. in length as it hatches from the egg; body a pale green, and head shiny black. The mature larva (fig. 2, B) is approximately 25 mm. in length; body a dark green, with two white lateral stripes down each side; head pale green, dotted with raised white tubercles, each giving rise to a short hair; anal

shield similarly covered with white tubercles, produced behind into two blunt, well-separated projections; body skin covered with a fine pubescence; prolegs on abdominal segments 3, 4, 5, and 6 and the normal anal prolegs; crochets of prolegs biordinal, arranged in longitudinal band.

BIOLOGY AND HABITS

There is but one generation of the pine butterfly yearly in Idaho. This fact, however, is rather confusing to the casual observer or collector, since there is a marked overlapping of the seasonal history events by individuals of this one brood. Elevation and exposure have a marked influence on the development of the insect. Imagoes, especially males, are often seen at high elevations as late as October.

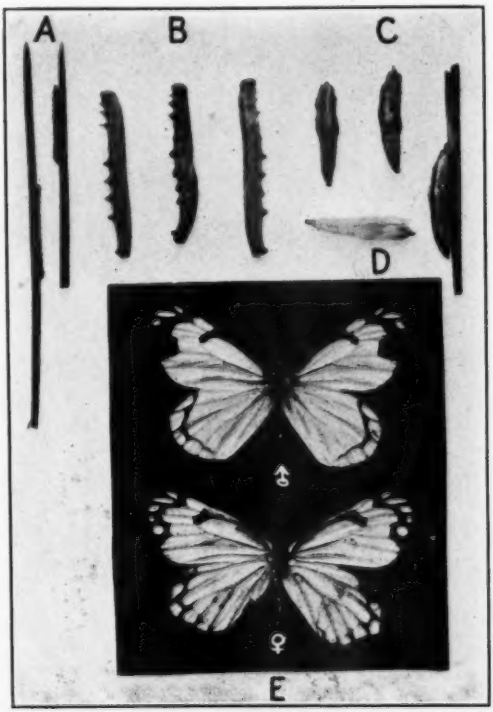

FIG. 2.—Pine butterfly: A, eggs; B, mature larvae; C, pupae; D, empty pupa case; E, imagoes

The overwintering eggs hatch about the time that the new needles begin to appear on the western yellow pine. This occurs during the first half of June, depending upon the season. The development of the young larvae is very slow during the first two weeks. They feed in clusters, encircling the needle, with their heads pointing toward the tip of the needle, making a tiny ring of black beads.

Only the fleshy part of the needle is eaten by the young larvae (fig. 3), but after the first molt the entire leaf is destroyed. During the first molt the shiny black head covering is shed, the subsequent color being a yellowish green. When the larvae are about half grown the habit of feeding in clusters is no longer continued, but often two or more may be found upon the same needle, especially if there is a shortage of food material. After the second molt growth is very rapid, and the larvae are approximately full-grown by the last of July, or about 50 days after hatching.

When mature, the larvae lower themselves by a silken thread, which sometimes is 75 feet or more in length, from the trees to the ground, where they pupate as chrysalids on shrubs, grasses, limbs, fences, tree trunks, or other objects. The pupal stage lasts from 15 to 20 days. Mating occurs almost immediately after the emergence of the adult, and oviposition takes place a few hours later. These eggs overwinter and hatch the following June.

The normal habit of the female is to deposit her eggs on the needles at the top of mature trees. However, in areas which have been severely defoliated she apparently is forced to seek suitable foliage on younger trees or else migrate to other areas, which she does in numerous instances.

FIG. 3.—Larvae of the pine butterfly feeding on the foliage of western yellow pine

NATURAL ENEMIES

Although the area defoliated by the pine butterfly in 1923 was at least 25 per cent larger than that in 1922, when the outbreak first became serious, there was a heavy mortality caused by the natural enemies of the insect, and it was believed that the epidemic was at

an end. This belief was fully substantiated during the 1924 season, as it was practically impossible to find a larva, and no damage was observed that season.

The most important of the enemies of the pine butterfly was a parasitic ichneumonid (*Theronia fulvescens* Cress.) (fig. 4). This insect lays its eggs on the caterpillars, which, though severely weakened, are for the most part able to reach the pupal stage. The adult (fig. 4, C) emerges in September. The parasitized pupae can easily be distinguished from the others by their dark brown color, the normal pupae ranging from a pale to a dark green. A predacious hemipteran (*Podisus placidus* Uhler) was also present in large numbers, but the importance of this insect in the reduction of the present epidemic is not known. It is possible that these parasites are responsible for the cessation of the many sporadic outbreaks of the pine butterfly which have occurred in the past.

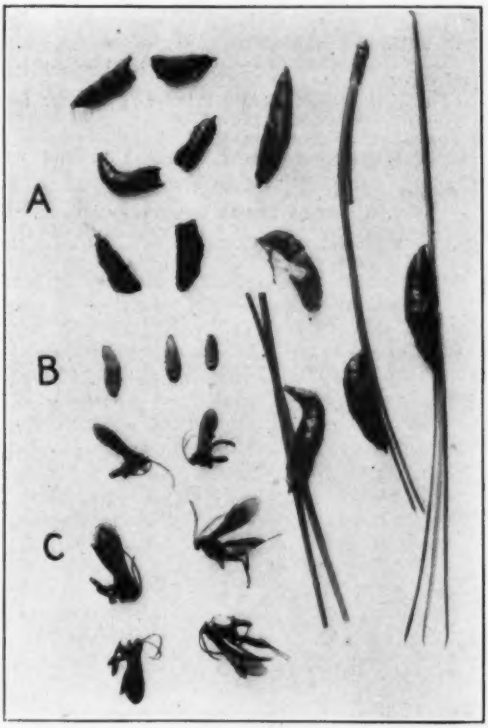

FIG. 4.—*Theronia fulvescens*, a parasite of the pine butterfly: A, parasitized pupal cases, some of which show emergence hole of parasite; B, larvae; C, imagoes

ECONOMIC IMPORTANCE

The economic importance of pine-butterfly epidemics is rather uncertain, as the degree of damage depends entirely upon the severity of the defoliation. Though the terminal buds were not injured by the insects and new needles were produced after the two years' defoliation, a large percentage of the overmature, decadent trees were unable to recover from the injury received and they began to die in 1924 and 1925. There is no doubt but that the effects of the damage which occurred during this outbreak will continue to appear for several years, with a loss ultimately amounting to several hundred thousand dollars.

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SUSCEPTIBILITY OF THE BEAN TO THE VIRUS OF SUGAR-BEET CURLY-TOP[1]

By EUBANKS CARSNER

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The disease of beans (*Phaseolus vulgaris*) of a mosaic type, which is caused by the virus of curly-top in sugar beets, has not been described so far as the writer is aware. The only reference that he knows of in this connection is one by himself,[2] in which the bean was reported as one of the species which had been tested and found nonsusceptible. This statement was made on the basis of a test with the Pink bean, a popular California variety. Interest in the matter was revived when circumstantial evidence suggested the possibility that a disastrous epidemic disease of beans which occurred in Twin Falls County, Idaho, in 1924 might have resulted from infestation of the beans by the leaf hopper *Eutettix tenella* Baker, which transmits the virus of curly-top.

When the question as to the cause of the bean disease in Idaho was raised, seed samples of seven of the varieties of beans commonly grown in Twin Falls County were obtained for the purpose of making test inoculations. Greenhouse plantings were made at once of two of these varieties—the Montana White, or Great Northern, and Prolific Black Wax—and later the plants were inoculated. Viruliferous leaf hoppers were caged on several plants of each variety, and nonviruliferous leaf hoppers were caged on similar plants as controls. The plants on which viruliferous insects were caged became diseased, while the controls remained healthy. Later, to prove that the diseased beans had curly-top, nonviruliferous leaf hoppers were caged on them and then transferred to healthy beets. The beets to which these insects were transferred developed symptoms of curly-top.

The symptoms of the disease on the beans were marked dwarfing and distortion of the leaves which grew out after infection had occurred. These affected leaves became darker green than were the corresponding leaves of the check plants, and became puckered, with their edges curved downward (fig. 1). The puckering was probably due, as is often the case in curly-top beets, to the severer checking of the growth of the fibrovascular bundles than of the intervening mesophyll. The leaf veins showed slight clearing when viewed by transmitted light, as is shown conspiciously by affected beets, but no irregular swellings such as are so characteristic of the disease in beets were found.

The greenhouse inoculations indicated a difference in susceptibility to the disease between the two varieties, the Montana White seeming more resistant. To test this matter further, field plantings

[1] Received for publication Jan. 4, 1926; issued August, 1926.

[2] CARSNER, E. SUSCEPTIBILITY OF VARIOUS PLANTS TO CURLY-TOP OF SUGAR BEET. Phytopathology 9: 413-421, illus. 1919.

were made of the two varieties mentioned, and also of five other varieties. Fifty to 75 plants of each variety were inoculated by caging four viruliferous leaf hoppers on each plant. One month after inoculation the following observations were recorded:

FIG. 1.—Curly-top of garden bean (*Phaseolus vulgaris*, var. Prolific Black Wax). Five viruliferous leaf hoppers were caged on the plant on the right on December 24, 1924. Five nonviruliferous leaf hoppers were caged on the plant on the left on the same day. (Photographed January 13, 1925)

The Montana White, or Great Northern, variety seemed rather resistant; the inoculated plants were only slightly smaller and less healthy in appearance than the uninoculated controls.

Prolific Black Wax was less resistant than Montana White, but was not as susceptible as the other five varieties.

Pencil Pod Black Wax was slightly more susceptible than Black Prolific.

Giant Stringless, Full Measure, and Improved Golden Wax seemed about equally susceptible and slightly more so than Pencil Pod Black Wax.

Black Valentine seemed most susceptible of all.

When the last observation was made on these field plants on September 17, about two-thirds of the plants in the row of Montana White were alive, and one plant was alive in the row of Prolific Black Wax, while in the rows of the five other varieties all plants were dead.

It is of interest to note in connection with these observations on resistance and susceptibility that, according to E. P. Brossard, county agent of Twin Falls County, the Montana White, as well as the Pink variety, was injured in decidedly less degree by the epidemic disease in that district than were other varieties, and that Black Valentine was the most seriously affected of all.

The writer is still uncertain whether the epidemic disease of beans in southern Idaho was curly-top or not. The epidemic was called to the writer's attention in the fall of 1924, and he had no opportunity to see affected plants in the field. However, certain lines of circumstantial evidence suggested that the disease might have been curly-top. One phase of this evidence which seems worth mentioning is the fact that the leaf hoppers invaded the cultivated areas of southern Idaho in enormous numbers in 1924; and the fact, observed by Severin [3] and by C. F. Stahl and the writer (unpublished), that when the leaf hoppers fly into cultivated areas in immense numbers they at first settle down on practically any green vegetation. Aside from this observation and the reported similarity in varietal resistance, it might be mentioned that the symptoms as described to the writer by Brossard and another observer corresponded to the curly-top symptoms seen when viruliferous leaf hoppers were caged on healthy bean plants. In the season of 1925 no serious damage to beans from disease was reported in southern Idaho. The writer has no direct evidence to support the view, but he is inclined to think that the nonoccurrence of the epidemic disease in that district in 1925 may be correlated with the fact that the number of the beet leaf hoppers was very much less in 1925 than in 1924, when there was a great abundance of them.

In regard to the occurrence of curly-top on beans in California, the writer has made no observations in commercial fields in that State. He is, however, authorized to quote the following statement made to him by Henry H. P. Severin, of the California Agricultural Experiment Station, who conducted studies in 1925 when the beet leaf hopper was exceedingly abundant in California:

> It was demonstrated that many varieties of field beans, as well as a large number of other crops, were affected with curly-top under natural conditions. In the lower Salinas Valley where Small White beans were grown in the vicinity of a beet field which had been plowed under owing to curly-top, the beet leaf hoppers flew into the bean fields, and an average of 63 per cent

[3] The following is quoted from a manuscript by H. H. P. Severin now being prepared: "San Joaquin Valley—Flights in middle valley: When the immense swarms of beet leaf hoppers flew into the cultivated regions on Apr. 14, 1919, they were found during the next day generally distributed on green vegetation, but later they congregated on their most favorable host plants for the purpose of feeding and egg laying."

of the bean plants showed typical symptoms of the disease on August 19–20. Bean plants showing curly-top symptoms were transplanted from the field to flower pots in the greenhouse. Noninfective beet leaf hoppers were allowed to feed on these bean plants for a period of three days or longer, and were then transferred to healthy sugar beets. In the cases where the beets developed curly-top, plainly the curly-top virus had been transmitted from bean plants naturally infected under field conditions. Noninfective leaf hoppers which had been allowed to feed on apparently healthy bean plants which had been transplanted from the field failed to produce curly-top in beets. Such leaf hoppers after feeding on healthy beans grown from seed in the greenhouse also failed to transmit the virus.

The question whether or not the leaf hopper finds the bean a favorable host plant is significant from the standpoint of the amount of damage to the crop that may be expected from curly-top. That the bean is not a favorable food plant for the insect is indicated by the fact that in cage experiments all of the leaf hoppers died within 17 days after being caged on the beans. Principally on this account the writer is inclined to the opinion that it is only in seasons when the leaf hopper is relatively very abundant that serious damage to the bean crop from curly-top may be expected, rather than that the disease will be a continual menace as it is to sugar beets.

QUANTITATIVE INHERITANCE IN PHASEOLUS[1]

By Karl Sax

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INTRODUCTION

The characters which involve productivity in plants are usually dependent upon so many factors that a satisfactory study of the inheritance and yield can be made only by a study of linkage relations with simple qualitative factors. This method of analysis should also show the interrelation of many of the factors involved in productivity.

MATERIALS AND METHODS

The parents selected for this investigation were the Symonds White bean, and a small-eyed bean selected from a cross of Improved Yellow Eye × Small White. They are both of local origin, and differ in many characters. The Symonds White bean, of unknown parentage, is very productive, comparatively early, and bears very large white seeds. The small-eyed bean is unproductive, bearing small, partially pigmented seeds. Although the Symonds is a bush variety the plants are very large and are as tall as those of the small-eyed variety, which is a runner type. Data on the parental varieties and the F_2 segregates are given in Table 1. The mean values of the various parental distributions were calculated from frequency distributions with smaller units in case of yield per plant and seed weight.

The F_1 was very productive; the plants were of the runner type, and the seeds were large, completely pigmented, and mottled. The F_1 and F_2 and the parental varieties were grown in an insect-proof cage to prevent cross-pollination. In the F_2 the date of appearance of the first blossom was recorded for each plant. The length and breadth of the largest leaf on each plant was measured in order to get an index of leaf type. Leaf type was obtained by dividing the length of the leaf by the width. The F_2 segregates were also described as runner or bush. All runner beans were trained on strings to permit maximum development. All beans on each plant were harvested, and the total yield in grams and average seed weight in centigrams were calculated later in the year.

EXPERIMENTAL DATA

The F_2 plants segregated in a ratio of 27 mottled: 9 self-colored: 12 eyed: 16 white, indicating that simple independent factors are involved for the characters pigmented versus white, completely pigmented versus eyed, and mottled versus self-colored. The segregation of vine type is also probably dependent on a single-factor difference which is not linked with the seed-coat characters described, although the proportion of bush-type plants is considerably too large.

[1] Received for publication Jan. 27, 1926; issued August, 1926. This is Paper No. 173 from the Biological Laboratory of the Maine Agricultural Experiment Station. Approved for publication by the director of the station.

The other characters—average weight of seed, total yield per plant, date of first bloom, and leaf type—did not show any simple segregation, but seemed to follow the usual type of so-called blending inheritance. The frequency distributions of these characters in the F_2, together with the parental distributions, are given in Table 1. With the exception of yield per plant, the F_2 distributions are more or less intermediate in the parental values. In the case of date of first bloom of the Symonds parent it is probable that the earliest plants were not recorded until they had been in bloom for several days, and accordingly the mean value should be somewhat earlier than recorded. In the case of yield per plant there is a very interesting behavior of the distribution in F_2. The mean value of the F_2 distribution is significantly greater than that of the more productive parent, and has a decidedly skewed frequency distribution. It is evident that these characters showed no simple segregation and that many factors are probably involved in effecting seed weight, yield per plant, date of bloom, and leaf type.

TABLE 1.—*Frequency distributions of quantitative characters in parents and F_2 of Phaseolus*

Plant	Number of plants in first bloom on—												Total	Mean
	July 11	July 13	July 15	July 17	July 19	July 21	July 23	July 25	July 27	July 29	Aug. 1	Aug. 3	Aug. 5		
Symonds				37	27	4	3	0	0	1				72	18.39±0.16
Eye				0	18	34	5	1	0	3				61	21.03± .19
F_2	3	3	22	119	96	64	24	8	0	6	0	1	1	347	18.98± .11

Plant	Number of plants whose leaf length divided by width gives index of—											Total	Mean
	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0		
Symonds	3	1	17	30	16	4	1					72	1.299±0.009
Eye					2	5	16	18	14	5	1	61	1.692± .011
F_2	1	2	10	57	96	75	71	24	8	0	3	347	1.47 ± .005

Plant	Number of plants giving yield per plant of the indicated weight in grams															Total	Mean
	0 to 5	5	10	15	20	25	30	35	40	45	50	55	60	65	70 to 75		
Symonds	1	7	22	27	12	3										72	15.57±0.41
Eye	6	45	9	1												61	7.49± .21
F_2	64	36	44	54	33	29	29	16	17	11	3	4	3	2	2	347	19.89± .55

Plant	Number of plants having seed weight as indicated in centigrams												Total	Mean
	20 to 25	25	30	35	40	45	50	55	60	65	70	75 to 80		
Symonds									18	40	13	1	72	66.65±0.27
Eye	27	33	1										61	25.08± .19
F_2		1	11	27	103	111	55	30	7	2			347	46.26± .24

In studying the association of size characters with qualitative characters it is impossible to distinguish between a single factor which effects both qualitative and quantitative characters and two separate factors which are closely linked. If two factors are involved, their linkage relations can be approximately determined by growing the F_3 families, as Sirks [2] has done in his study of the inheritance of seed weight in the garden bean. So far as the study of the inheritance of size and yield characters are concerned, however, it makes little difference whether a single factor effects both qualitative and quantitative characters or two linked factors are involved. The relative differences between each homozygous class of F_2 segregates and the heterozygous class will also be the same regardless of the amount of crossing over between the two factors. In the following discussion we have assumed that the size factors are linked with the qualitative factors when the respective characters are associated.

If these quantitative characters are dependent upon a number of genetic factors for their expression, one would expect to find some linkage with one or more of the four independent characters involving seed-coat pattern or vine type. The data showing the relation between the qualitative and quantitative characters are shown in Table 2. In previous investigations it was found that seed weight was linked with mottling and pigmentation of the seed coat.[3] In this cross there seems to be no linkage between seed weight and seed-coat pattern, although the difference between the parental varieties is very great.

TABLE 2.—*Data showing segregation and relation of qualitative and quantitative characters in F_2 of Phaseolus*

Segregation in F_2	CLASSES OF SEGREGATES					
	Mottled	Self	Eyed	White	Total number of plants	
Numbers obtained	142	46	80	79	347	P=.12
Theoretical for 27: 9: 12: 16	145	49	65	86		
Vine type:						
Runner	89	35	56	56	236	
Bush	53	11	24	23	111	
					Runner	*Bush*
Seed weight	46.63±0.40	46.11±0.62	45.70±0.47	46.25±0.47	47.09±0.28	44.50±0.43
Yield	21.46±1.00	21.41±1.35	17.44±1.01	18.67± .90	24.21± .67	10.70± .61
Date of bloom	18.32± .16	19.96± .36	19.88± .25	18.70± .16	19.25± .13	18.42± .42
Leaf type	1.452± .008	1.502± .012	1.486± .013	1.466± .011	1.480± .006	1.452± .016

Yield per plant has also been found to be linked to some extent with seed-coat characters.[4] In this cross the mottled and self-colored segregates are significantly more productive than the eyed and white segregates. This suggests that one or several of the factors for productivity are linked with the factor for extension of the pigment. If this is the case, then the white segregates should be more or less intermediate in productivity as compared with the mottled or self

[2] SIRKS, M. J. THE INHERITANCE OF SEEDWEIGHT IN THE GARDENBEAN (PHASEOLUS VULGARIS). 1. Genetica 7:119-169, illus. 1925.
[3] SAX, K. THE ASSOCIATION OF SIZE DIFFERENCES WITH SEED-COAT PATTERN AND PIGMENTATION IN PHASEOLUS VULGARIS. Genetics 8: 552-560. 1923.
[4] SAX, K. THE NATURE OF SIZE INHERITANCE. Natl. Acad. Sci. Proc. 10: 224-227. 1924.

classes and the eyed segregates, because one-fourth of the whites should be homozygous for the extension factor and one-half of the whites should be heterozygous for the extension factor. The white segregates are more or less intermediate in yield, as would be expected, although the differences are not statistically significant. However, the odds are greater than 30 to 1 that the mottled and self classes are more productive than the eyed and white classes.

In calculating the probable errors of the differences in weight or yield of the various classes of segregates the formula $P.\ E._1^2 + P.\ E._2^2$ was used, although this method is legitimate only when there is little or no correlation between the two distributions which are being compared.[5] For instance, it would be possible to obtain a fairly high correlation between the two classes of segregates if the plants were grown on heterogeneous soil. Both classes of segregates would be relatively productive on a good soil and relatively unproductive on a poor soil. By comparing adjacent or neighboring segregates, the degree of soil variability can be determined even when the plants themselves are not systematically distributed so that the method of testing soil heterogeneity suggested by Harris [6] can be used. In the comparison of the mottled and self segregates versus the eyed and white classes, the yield of each plant was correlated with the yield of the nearest contrasted segregate. This method gives an approximate test of soil heterogeneity, which is measured by the value of r. In this cross the value of r was 0.09 ± 0.07, indicating that there was no significant correlation between the two classes of segregates under comparison. Accordingly, then, the probable error of a difference, in this case, can be taken as the square root of the sum of the squares of the probable errors of the two frequency distributions.

Evidently the time of flowering is determined by a number of factors, and might be expected to show linkage with one or more of the three seed-coat characters. As indicated in Table 2 the self-colored and eyed segregates bloomed significantly later than either the mottled or white classes. There is, however, no consistent superiority of one class of segregates. High productivity can not be linked with the recessive mottling factors, because most of the eyed segregates are mottled. It is possible that certain combinations working together result in productive or unprofuctive plants. The odds are about 150 to 1 that either the mottled or the white class blooms earlier than eigher the self or eyed segregates, but time of flowering does not seem to be linked with any single one of the seed-coat characters under consideration.

The leaf types of the two parents differ considerably and show no evidence of simple segregation in the F_2. There is not as much association between leaf type and seed-coat characters as might be expected. The leaves of the self-colored segregates are significantly different in shape than the mottled segregates, but when compared with the other two classes of segregates the differences are of doubtful significance.

The association of quantitative characters with vine type is especially interesting. The seed weight of the runner segregate is greater than that of the bush type, although in the parents this relation is

[5] SAX, K. THE "PROBABLE ERROR" IN HORTICULTURAL EXPERIMENTS. Amer. Soc. Hort. Sci. Proc. (1924) 21: 252-256. [1925.]

[6] HARRIS, J. A. ON A CRITERION OF SUBSTRATUM HOMOGENEITY (OR HETEROGENEITY) IN FIELD EXPERIMENTS. Amer. Nat. 49: 430-454, illus. 1915.

reversed. The relation between seed weight and type of vine may not be due to linkage in the usual sense of the term, but it may be the result of greater vigor of the runner segregate.

The runner segregates in F_2 were more than twice as productive as the bush segregates, although the bush parent was about twice as productive as the runner parent. The runner segregates were also much more productive than the productive bush parent. This reversed relation between yield and parental characters can hardly be explained entirely on the basis of linkage of productivity factors with the indeterminate type of growth. There might, of course, be some such linkage, and yet the runner parent would be relatively unproductive, owing to numerous factors for low yield in other linkage groups. In this case, however, the yield of the F_2 runner segregates was so much greater than the yield of the runner parent that there must be some other explanation for the increased yield in the F_2. The runner parent was relatively small and weak and was no taller than the Symonds bush parent, a very vigorous, productive type of bush bean. In the F_2 it is probable that the factor for vigor and productivity contributed by the Symonds parent, combined with the runner type of vine which increases the potential bearing surface of the plant, resulted in F_2 segregates of unusual productivity. Although the eyed parent was of the runner type and should have the capacity to carry a large number of seeds, yet it was so lacking in vigor that it produced comparatively few pods.

The differences in date of bloom of the bush and runner segregates are statistically significant and are in accord with the parental relations. It is quite possible that the relation between vine type and time of flowering is due to linkage in the usual sense of the term, although perhaps the greater vigor of the runner type might cause the delay in blossoming. There is no significant difference of runner and bush leaf types in the F_2. Seed weight and date of bloom show some association or linkage with vine type, but the association is opposite that found in the parents. Such a relation might be expected, occasionally, of course, but it is more probable that the association in this case is due to physiological causes.

SUMMARY

In a cross of Symonds white bean × a small-eyed bean of local origin, the F_2 segregates gave a ratio of 27 mottled, 9 self-colored, 12 eyed, 16 white, indicating that the simple factors for pigmentation, mottling, and extension of the seed-coat pigment are independent. Vine type, whether runner or bush, is also probably dependent upon a single factor which is independent of the above seed-coat characters. The characters, date of bloom, leaf type, seed weight, and yield per plant, apparently are dependent on multiple factors in each case. If these quantitative characters are dependent on many independent factors for their expression, there should occasionally be some linkage of these characters with the simple qualitative characters of seed coat and vine type. There is some linkage of yield with the extension factor. Date of bloom and leaf type also show some association with certain seed-coat characters, although the relation is not a simple one. Seed weight and

date of bloom show some association or linkage with vine type, but the association is opposite that found in the parents.

The association of yield per plant with vine type is of considerable interest from the standpoint of the plant breeder. In the F_2 the runner segregates are more than twice as productive as the bush segregates, although in the parents this relation is reversed. The runner parent is very weak and unproductive, while the bush parent is vigorous and productive. In the F_2 the combination of vigor and potential productivity with the runner type of vine which permits a maximum development of pods, results in runner segregates more than three times as productive as the runner parent and much more productive than the bush parent.

CRANBERRY FLOWER-BUD INVESTIGATIONS [1]

By DONALD SEWALL LACROIX

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INTRODUCTION

The significance of the study of the fruit-bud development in any plant of economic importance is apparent when it is considered that the subsequent fruitfulness of any such plant depends upon the formation and proper development of the buds in their incipient stages. To quote Drinkard,[2] "If it is possible to ascertain the time of the formation of the fruit buds of our leading fruits, and to trace the development of the buds through every stage of their life history, noting the time of every important change, this information will enable us to attack the still more vital question of the *factors* which influence the formation of fruit buds and are conducive to their proper development."

The study of buds through their early stages is a rather recent phase of horticultural research, the most important of such investigations having been conducted since the late nineties. Fruit growers have recognized for many years that the buds of our important fruits are formed some time during the season previous to blossoming. Pomologists of many of the experiment stations have worked out in detail the life histories of the fruit buds of practically all of the most widely grown fruits, it now being known just when bud formation takes place and when the important changes come about in the subsequent development of the buds.

In 1901 Goff [3] reported on a few preliminary studies of the cranberry bud in Wisconsin. Since that time no work has been recorded indicating similar studies of this plant. In view of this and of the fact that cranberry growing is one of the important industries in Massachusetts, it was deemed advisable to investigate and bring together observations on the life cycle of the cranberry blossom.

OUTLINE OF THE WORK

The bud studies were conducted by the author on Massachusetts bogs. It is a general practice in the Cape Cod district to flood the cranberry bogs with fresh water during the winter. This winter flowage may be held on the bogs until late in May, as a control measure against certain insect pests; or it may be drained off early in April. Here a problem presents itself. What effect has early drainage on the development of the cranberry bud and the subsequent development of the fruit, as against late holding of the winter flowage?

[1] Received for publication Jan. 18, 1926; issued August, 1926. Submitted for publication with the approval of the Director of the Massachusetts Agricultural Experiment Station.

[2] DRINKARD, A. W., JR. FRUIT-BUD FORMATION AND DEVELOPMENT. Va. Agr. Expt. Sta. Ann. Rpt. 1909/10: 159–205, illus. 1911.

[3] GOFF, E. S. INVESTIGATION OF FLOWER-BUDS. Wis. Agr. Expt. Sta. Ann. Rpt. 18: 306–310, illus. 1901.

The studies, carried on through three complete seasons, have been divided into two parts: (1) A study of the morphology of terminal buds taken throughout the year; and (2) blossom counts and berry counts in the field. In each case an attempt has been made to note any differences on "early drained" bogs and "late-held" bogs, to determine just what effect early draining or late holding of winter flowage has on the buds, blossoms, and set of fruit.

MORPHOLOGY AND LIFE HISTORY OF THE CRANBERRY BLOSSOM

Material to be used in a study of the morphology and life cycle was collected on the experiment station bog at East Wareham at two-week intervals through summer and fall; at monthly intervals during the winter; at two-week intervals after April first; and semi-weekly immediately before the blossoming period. Samples were taken from each of the two most widely grown varieties, Early Blacks and Howes.

FIG. 1.—Types of cranberry terminal buds: A, Terminal bud containing incipient fruit buds; B, terminal bud of "rosette" type; contains no incipient fruit buds; C, terminal bud which develops into new growth with no fruit buds. All × 8.3

Each sample consisted of 25 terminal buds, and was subjected to the usual process of killing in chrom-acetic acid and washing. The material was then carried through the alcohols to 70 per cent in which it was preserved until winter, when the embedding, sectioning, staining, etc., were completed in the laboratory.

The cranberry vine, as it trails along over the surface of the bog, sends up woody stems or uprights. The stems may or may not bear terminal buds. The terminal buds, when well developed, may be one of these three types: (1) A round, compact, solid bud, covered with tightly fitting scales (fig. 1, A); or (2) a loosely set rosette of scales and small leaves (fig. 1, B); or (3) an elongate, sharp-pointed, scale-covered "shoot bud" (fig. 1, C). The first type contains blossom buds. The second contains no blossom buds, it usually developing as a result of some injury to the normal terminal buds. The third type usually develops into a new growth of upright, and contains no embryo flowers. These three types are usually distinct by the first of October. Prior to that time it may be difficult to distinguish between a terminal bud of the first type and one of the last, since they assume similar shapes early in the season. It is the first type which is dealt with in this paper.

The photomicrograph of the longitudinal section of a terminal bud shown in Figure 2, A, represents the general condition of buds late in July and early in August. The bud in Figure 2, A, was taken August 1, 1924, and it is representative of buds taken during the two previous years on that date. Under the bud scales there is a "crown" or growing point of meristematic tissue. Incipient bud scales are present as small lateral outgrowths. No flower-bud primordia are yet visible.

Figure 2, B, is representative of sections of buds taken in the middle of August, the figure shown being from a bud taken August 15, 1924. An incipient flower bud has started as a minute lateral outpushing, as may be seen at the right of and just below the growing point. The earliest embryo flower bud on the station bog was found on August 10, 1922. Goff [4] states that he found the first incipient flowers in Wisconsin on September 16.

Figure 2, C, is representative of buds taken from late August to the middle of September. The incipient flower buds have become larger, and lateral projections around the bud on the left are the first intimation of petals. These projections are more pronounced in Figure 2, D, which is typical of buds in middle September and early October. In this bud the rudimentary sepals can be seen immediately outside of and below the developing petals.

In October growth is apparently limited to the petals and sepals, an incurving and overlapping of the immature petals taking place. It is in this condition that the buds pass the winter, and they are thus doubly protected by the bud scales and by the overlapping petals (fig. 2, E).

Growth is resumed soon after the removal of the winter flowage; it is manifested in the terminal bud by the formation of two protuberances beneath the incurved petals (see bud at right in fig. 2, F). It is at this point that the tissues for the future stamens are first laid down as visible thickenings, in the formation of these two protuberances, which are the primordia of the stamens. Similar buds have been taken on late-held bogs where the water was shallow and warm, but as a general rule this type of growth is found only after the winter flowage has been off for a week or more. Up until this time, there has been no marked change in the outward appearance of the terminal bud.

In Figure 2, G (bud taken May 3, 1923) the conditions existing late in April to early in May are shown. Here the small flower buds are no longer sessile but are distinctly stalked, the petals and sepals are more elongate, and the primordia of the stamens are somewhat larger. In the bud at the right a central prominence beneath the immature stamens indicates an incipient stage of the future pistil. A distinct swelling and rounding out of the whole terminal bud has become apparent, and the first indications of "bursting" are visible. The bud scales are no longer wrapped tightly over the bud, but are beginning to separate. Figure 3, A, shows an upright bearing a terminal bud in this stage.

Figure 2, H, which is representative of buds taken in the middle of May, shows clearly (at the left) two of the four or five proturberances which later become elongate and fuse to form the pistil.

[4] Goff, E. S. Op. cit.

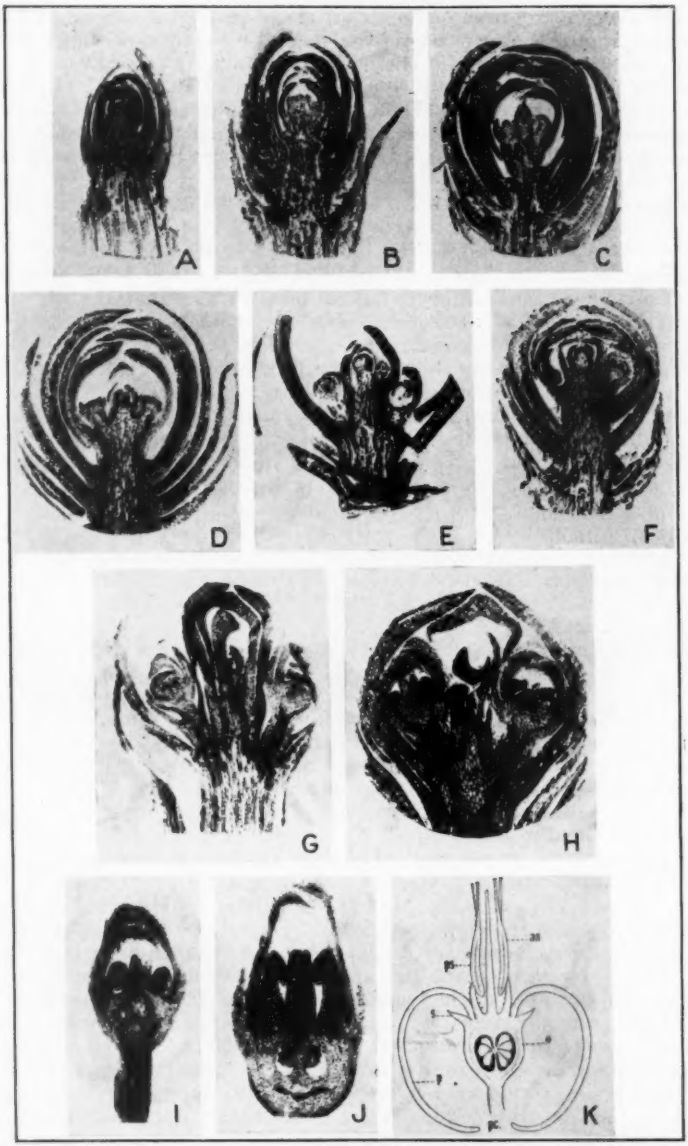

FIG. 2.—Axial longitudinal sections of terminal buds, flower buds, and mature flower, of cranberry, taken at different stages of development: A, Terminal bud taken August 1; B, terminal bud taken August 15; C, terminal bud taken August 20; D, terminal bud taken September 20; E, terminal bud taken October to April; F, terminal bud taken April 17; G, terminal bud taken May 3; H, terminal bud taken May 17; I, terminal flower bud taken May 27; J, terminal flower bud taken June 9; K, diagrammatic sketch of mature flower. (*an*, anther; *ps*, pistil; *pc*, pedicel; *s*, sepal; *p*, petal; *o*, ovary.) (A to J, inclusive, × 20)

Bud conditions during the latter part of May are represented in Figure 2, I (bud taken May 27, 1923). This photomicrograph was taken of an immature flower bud which had become wholly independent of the protective bud scales. It had reached the "rough-neck" stage, so called because the small flower buds are scattered closely along the new shoot, giving the latter a rough appearance (fig. 3, B).

Figure 2, J (a photomicrograph of a bud taken June 9, 1923), represents the general condition of buds shortly before blossoming, a stage that is sometimes referred to as a "pod" (fig. 3, C and D). The immature ovary is distinct, and the seed primordia (ovules) can easily be observed hanging as clusters in the cavity. The fusion of the carpels to form the pistil is well toward completion. The petals are more elongate and are arching upward and outward prior to opening. A slightly more advanced condition is indicated in Figure 3, D, and E.

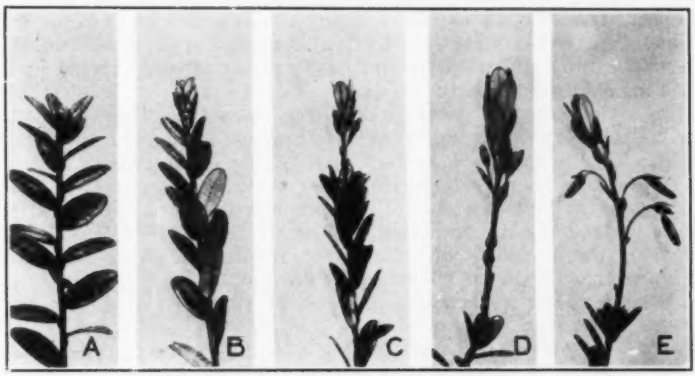

FIG. 3.—Upright, or shoot, of cranberry, showing buds in progressive stages: A, Terminal bud just bursting open; B, an upright in so-called "rough-neck" stage; C and D, flower buds in the "pod" stage; E, flower buds drooping just before opening. (All natural size)

A diagrammatic sketch of the mature cranberry flower in longitudinal section is shown in Figure 2, K.

These illustrations show the complete life cycle of the cranberry flower. In each case an attempt was made to pick out samples of material that were fairly representative of conditions at the various periods. There is more or less overlapping. For example, in some instances buds were found passing the winter in the condition represented in Figure 2, F; but such a case was not a common one and could not well be called a fair sample of conditions in the winter.

FIELD OBSERVATIONS

Many growers have held the opinion that, although late holding of winter flowage on cranberry bogs is beneficial in controlling certain insect pests, such practice has a tendency to reduce flower-bud development and consequently to reduce the yield. As far as can be determined all arguments advanced on either side of the question have not heretofore been substantiated by actual tests. In view of this fact it was considered desirable to collect data on bud conditions from a large number of bogs over a period of several years, and in

this way try to arrive at more definite answers to the various questions raised in connection with late holding of winter flowage.

For three seasons the writer recorded dates of water removal from 16 bogs in southeastern Massachusetts, and classified them into two groups: (1) "Early drained" bogs, or those drained of the winter flowage on or before April 15, and (2) "late-held" bogs, those kept under full winter flowage until May 20 or later.

In the spring of 1923 bud counts were taken on bogs of each group. One hundred terminal buds were collected from each bog in April. These buds were cut transversely and examined with a hand lens to determine whether immature flower buds were present. The study was repeated in May after water removal, and it was concluded that late holding of the winter flowage did have a retarding influence on bud development.

Upon the removal of this flowage late in May, growth is resumed and development advances with great rapidity, the blossoming period being not more than a few days later on late-held bogs than on early drained ones. Buds exposed by late drainage are not subjected to the setbacks of cold weather and heavy frosts of early spring as are the buds exposed by early drainage.

The development of the buds of Howes closely parallels that of the Early Blacks until late spring when the development of the Howes advances less rapidly than does that of the other variety. There may be a difference between the two varieties of from 5 to 10 days in the dates of first blossoms. The incipient flowers in either variety are in evidence by the middle of August.

During the period of bloom, counts were made on these bogs of the number of blossoms per hundred uprights. To do this, 100 uprights were taken at random on each of two ends of a bog, from the two most commonly grown varieties, Early Blacks and Howes. The blossom counts are given in Table 1, the figures being the averages for each season of all early drained and late-held bogs.

TABLE 1.—*Counts of cranberry blossoms and berries on uprights taken at random from bogs, from Early Blacks and Howes varieties, the two most commonly grown in the Cape Cod section*

Year	Number of blossoms per 100 uprights		Number of berries per 100 uprights			
			Howes		Blacks	
	Howes	Blacks	Set	Not set	Set	Not set
EARLY DRAINED BOGS						
1923	351.5	414.6	158.0	252.2	163.5	232.5
1924	320.4	300.0	135.2	116.0	149.8	127.0
1925	281.0	260.0	154.0	135.2	163.0	92.5
Average	317.6	324.8	149.0	167.8	159.0	150.6
Per cent set			46		51	
LATE-HELD BOGS						
1923	304.0	300.8	189.6	150.3	160.4	133.4
1924	302.0	248.0	160.8	100.0	123.3	111.3
1925	279.0	301.0	162.0	109.6	178.0	121.5
Average	295.0	283.2	170.8	119.9	153.9	122.0
Per cent set			59		56	

The average for the three seasons indicates that late holding may be responsible for a reduction in the number of flowers. For example, for the three years the average number of flowers per 100 uprights of Howes was 317.6 on early drained bogs and 295.0 on late-held bogs.

Flowers were aborted in some instances. That is, the two leaflets or bracts which always accompany a blossom could be found on some uprights, but no floral development took place, no blossom was formed. This condition was observed in 1923, but in such small numbers that it apparently had no important bearing on the question. No such condition was found in 1924 and 1925. Table 2 indicates the average number of blossoms per 100 uprights, as compared with the average number of aborted blossoms, on the two types of bogs. The figures therein indicate that late holding increases the tendency of blossom abortion.

TABLE 2.—*Average number of blossoms and average number of aborted blossoms per hundred of uprights on Early Blacks and Howes, the two varieties of cranberries most commonly grown in the Cape Cod section, on the two types of bogs*

1923	Number of blossoms per 100 uprights			
	Howes		Blacks	
	Aborted	Perfect	Aborted	Perfect
Early drained bogs	4.75	351.5	0.66	414.6
Late-held bogs	11.00	304.0	10.40	300.8

Counts were made in similar manner of the number of berries which set and of the number of "berryless" pedicels (to indicate the "nonset"). These averages are included in Table 1 also. These data indicate that late holding of winter flowage slightly increased the set of Howes berries and very slightly decreased the set of Early Blacks. On the other hand, the number of berries which did not set is fewer in both varieties on the late-held bogs. By adding the number of berries that set and the number of those that did not a sum is derived which may be called the "possible set." Using this as a basis, the percentage set is readily obtained. Forty-six per cent of the Howes set on early drained bogs, and 59 per cent set on the late held. Fifty-one per cent of the Early Blacks set on early drained bogs, and 56 per cent set on the late held. The percentage set was greater on late-held bogs in both varieties. This indicates that while the actual number of blossoms and the actual number of berries may be decreased by late holding, the percentage of berries which set may be increased.

From a practical standpoint, then, late holding of the winter flowage reduces bloom, but increases the set of fruit. Meeting the requirements of a large number of blossoms is such a heavy drain on the energy of a plant that a greater percentage of the latter do not become fruit. On the other hand, if the number of blossoms is reduced through the influence of external factors (late holding) the plant energy has only to supply the requirements of a few blossoms—hence the increased ability to set fruit.

Further investigations on weather conditions (rainfall, temperature, etc.) resulted in but one satisfactory set of correlations, namely,

temperature and yield. The mean annual temperatures for Cape Cod were obtained from records at the meteorological stations at Middleboro, Plymouth, Hyannis, and Provincetown, by averaging the mean annual temperatures of these four stations. The total crop in barrels for each year was compared with this mean annual temperature. The yield was considerably greater in a year following a relatively warm year, and correspondingly smaller in a year following a cool year. See Figure 4.

For example, 1913 was the warmest year in the period from 1902 to 1919, inclusive, and the crop of 1914 was the largest in the period. The years 1904, 1916, and 1917 were the coolest of the period and the crops for 1905 and 1917 were the smallest.

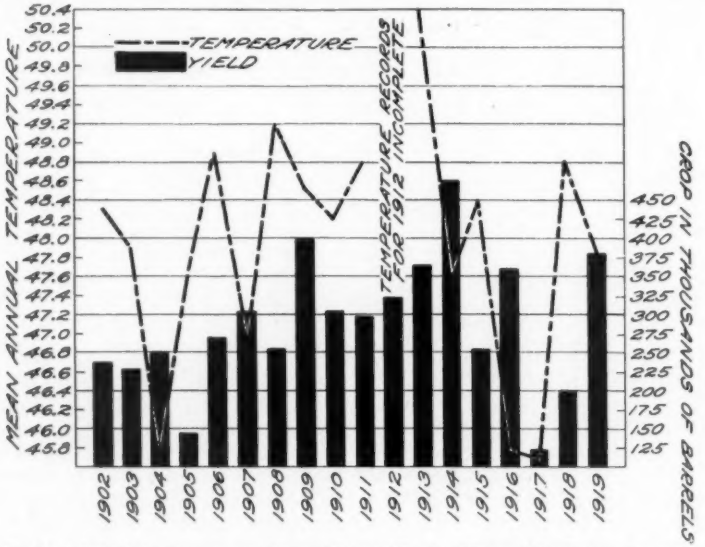

FIG. 4.—Relation between mean annual temperature and the size of the annual cranberry crop, on the Cape Cod, Mass., bogs, for the 18-year period 1902 to 1919, inclusive

It would seem, then, temperature is an important factor in fruit-bud formation. Crops can not be harvested unless fruit buds are formed, and fruit buds apparently require a warm season for the best development.

SUMMARY

Flower buds are first observed on cranberry plants on Massachusetts bogs in the middle of August of the season previous to blossoming.

A process of growth and development is observed in the incipient flower buds through the late summer and early fall preceding blossoming.

Apparently no growth that is visible takes place in the buds during the winter.

Growth is resumed in the spring immediately after the removal of winter flowage, and continues at a rapid rate until full bloom in June.

The field observations indicate that late holding of winter flowage tends to retard bud development, decrease the number of blossoms, and increase the percentage set of fruit.

The largest crops of cranberries are harvested the year following a high mean annual temperature, and the smallest crops are harvested the year following a low mean annual temperature. Since the flower buds are formed and partly developed the year previous to blossoming, it appears that temperature is an important factor in the formation and development of the flower buds, a high temperature being favorable to their growth, a low temperature unfavorable.

BROWN-SPOT DISEASE OF SOY BEAN [1]

By FREDERICK A. WOLF, *Plant Pathologist, Office of Fruit Diseases, Bureau of Plant Industry, United States Department of Agriculture,* and S. G. LEHMAN, *Plant Pathologist, North Carolina Agricultural Experiment Station*

INTRODUCTION

The investigations reported in this paper were begun late in the summer of 1922, when the writers' attention was first drawn to a leaf-spot disease of soy bean, *Soja max* (L.) Piper, in North Carolina with which a species of Septoria was associated. The crop was at that time practically mature, and considerable defoliation, beginning with the lowermost leaves, was in progress. During the following season the disease was epiphytotic in character, presumably as the result of excessive rains; two protracted rainy periods occurred during that year, one in June and the other in late August and early September, with much damp, warm weather in the interim.

It was evident early in the course of these studies that the disease was very probably identical with one which had first been described in 1915 in Japan by Hemmi [2] under the appropriate common name of "brown-spot disease." Specimens of this disease were first collected in the Province of Kitamai in September of the previous year by K. Miyabe. Hemmi's report [3] shows that it was very common and very widely distributed in 1914 throughout Hokkaido, in the Provinces of Ishikari, Tokachi, and Iburi. It was present that year in every field and was the cause of very considerable damage. In 1915 S. Ito collected it in Honshu in the Province of Echigo. In all probability brown spot has existed in Japan for years, as pointed out by Hemmi, and it occurs in other parts of Asia where soy beans are grown. Miura [4] observed it in Manchuria, and states that it very probably occurs also in Chosen (Korea). Through the kindness of K. Nakata, Kyushu Imperial University, Fukuoka, Japan, the writers received an unnamed collection made June 18, 1914, by S. Takimoto at Suwon, Korea, which proved to be the brown-spot disease. Brief statements by the writers in the Annual Reports of the North Carolina Agricultural Experiment Station for 1923 and 1924 [5] appear to constitute the only records of the occurrence of this disease within the United States. It no doubt occurs in other States where soy beans are grown, but the only definite information on this point is contained in correspondence with J. F. Adams, who noted it in Delaware in 1923.

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[1] Received for publication Jan. 9, 1926; issued August, 1926.

[2] HEMMI, T. A NEW BROWN-SPOT DISEASE OF THE LEAF OF GLYCINE HISPIDA MAXIM. CAUSED BY SEPTORIA GLYCINES SP. N. Sapporo Nat. Hist. Soc. Trans. 6:12-17. 1915.

[3] HEMMI, T. Op. cit.

[4] MIURA, M. [DISEASES OF THE MAIN AGRICULTURAL CROPS OF MANCHURIA.] Agr. Expt. Sta. So Manchuria Ry. Co. Bul. 11, 56 p., illus. [In Japanese. English abstract in Japan. Jour. Bot. 1: (9), 1923.] 1921.

[5] WOLF, F. A. REPORT OF THE DIVISION OF PLANT PATHOLOGY. N. C. Agr. Expt. Sta. Ann. Rpt. 46: 92. [1923.]

——— and LEHMAN, S. G. REPORT OF DIVISION OF PLANT PATHOLOGY. N. C. Agr. Expt. Sta. Ann. Rpt. 47: 83. [1924.]

SYMPTOMS OF BROWN SPOT

The disease is most noticeable upon the foliage, where it is characterized by brown or reddish-brown angular spots 2 mm. in diameter. It is primarily a leaf-spot disease and causes severe defoliation. It appears also on the stems and pods as the plants approach maturity.

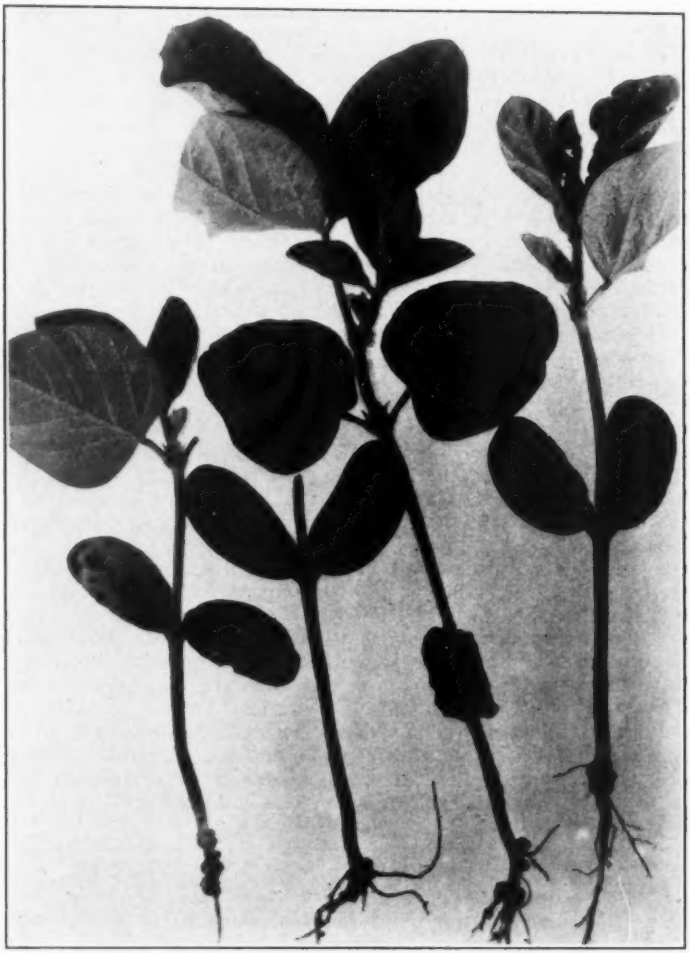

FIG. 1.—Seedling soy beans with brown-spot lesions on cotyledons and first true leaves

The first evidence of disease is apparent early in the season, when the first pair of true leaves have formed. At this time there are irregular dark-brown patches, varying in size from minute specks to areas 4 mm. in diameter, on the cotyledons (fig. 1). Before the cotyledons have shriveled and fallen, infections have become apparent

on the unifoliate or first true leaves. The lesions on these leaves are conspicuously reddish brown on both leaf surfaces. They are angular in outline, being limited by the small veins, and vary in size from 1 to 5 mm. in diameter (fig. 2). The larger ones arise from

FIG. 2.—Characteristic angular, reddish-brown lesions on unifoliate leaves

coalescence of adjacent spots. The tissues surrounding the diseased areas are pale green at first and then they become decidedly chlorotic, after which the leaves fall off.

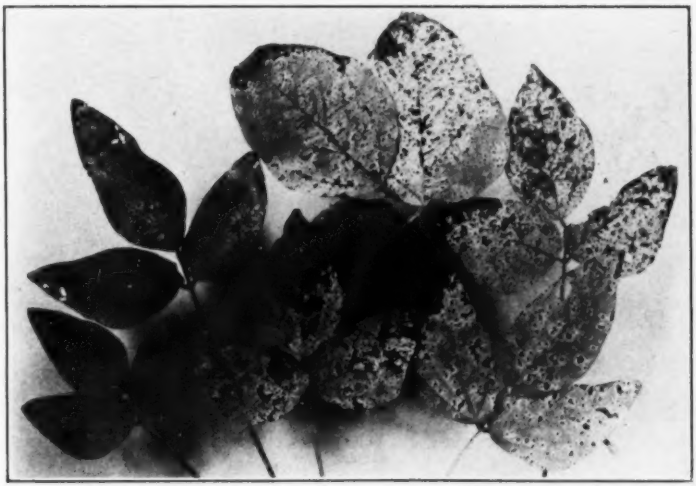

FIG. 3.—Mature leaves showing abundance of lesions, chlorosis, and dead leaf tips

The disease next involves the trifoliate leaves, upon which the spots become more numerous as the season advances and, by confluence of the spots, large, light-brown, irregular areas are formed (figs. 3 and 4). The color gradually deepens until the diseased areas are

dark brown to blackish brown, which appears to be intensified with the loss of the normal green of the intervening tissues. The disease progresses upward on the plants from the lower leaves. By late summer, if conditions are favorable for the development and spread of the causal organism, the older leaves become so spotted that it is difficult to distinguish individual lesions. At this time each leaflet

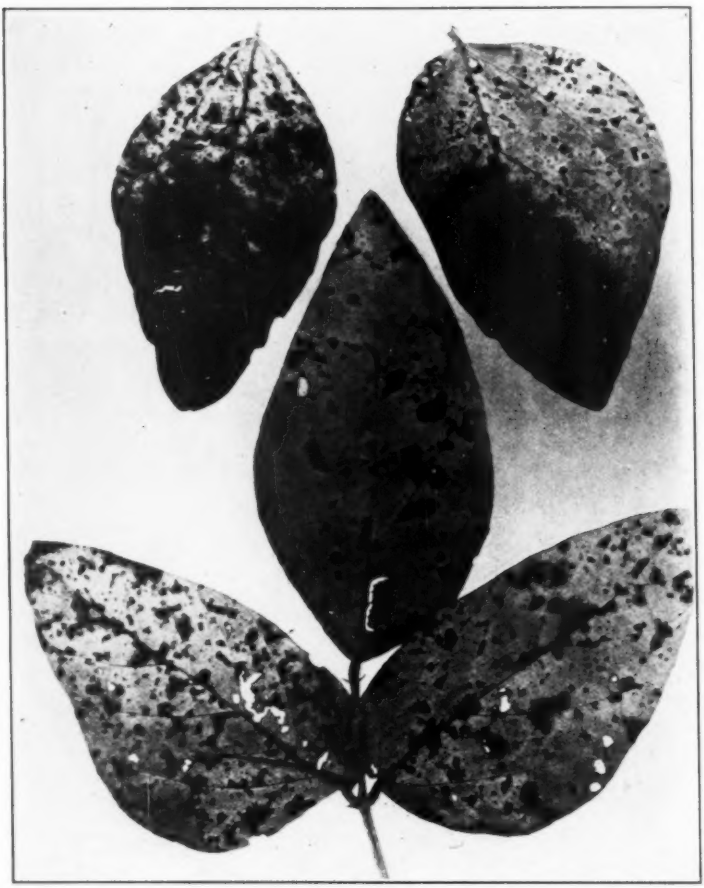

FIG. 4.—A single trifoliate leaf affected with brown spot, and two leaflets with dead tips which would soon have fallen

may bear thousands of specks, with no distinctive microscopic feature except their rusty brown color (fig. 5). Such leaves are prematurely shed from below upward.

The disease on the stems manifests itself by the presence of indefinitely margined, brown discolorations. These lesions vary in size from tiny specks to areas several centimeters in length, and they

may nearly or completely encircle the stems. Tissues adjacent to these discolorations are less green than normal tissues, and as chlorosis advances the diseased areas become more conspicuous (fig. 6). Main stalks, branches, and leaf stalks are all involved in the same manner.

The spots on pods are similar in all respects to those on stems The smaller areas are pinpointlike, whereas the larger ones may come to involve more than half the surface of the pods (fig. 7).

FIG. 5.—A single leaflet in late summer, when the lesions are so abundant that irregular brown patches are formed by confluence of adjacent spots

THE BROWN-SPOT FUNGUS

ISOLATION

Mature lesions on leaves, stems, and pods bear innate, brown pycnidia, and each source has been found to serve equally well as the others in isolation trials. The fungus may readily be isolated when tissues bearing pycnidia are macerated in sterile water and drops of the suspension are spread over the surface of hardened-agar plates. Conidial germination occurs promptly, but it is two or three days before the colonies become visible to the unaided eye. The colonies are olive brown from the first, they develop slowly and remain small. Within three weeks they reach a diameter of 5 to 10 mm. (fig. 8). The mycelium is dense and stromalike. Pycnidia form in abundance on such media as potato cylinders, potato agar, steamed corn meal, steamed tapioca, and sterile bean pods.

MORPHOLOGY

Lesions on stems and leaves sectioned in paraffin and stained show that the pycnidia are immersed and open to the surface by a large pore (fig. 9, D, E). Those in the leaves are globose to conico-globose,

and open mainly to the upper leaf surface. Those on the stems are flattened, because of their position between mechanical tissues. They commonly measure 90 to 100 μ in diameter, varying from 60 to 125 μ. They are either scattered or quite densely gregarious, and when two are sufficiently close they are flattened on the sides in contact. The pycnidial wall is thin and membranaceous (fig. 9, E).

FIG. 6.—Brown spot on stems and branches

The conidia are hyaline, filiform, curved, and indistinctly 1 to 3 septate. The length varies between extremes of 21 and 50 μ; but most of them are 35 to 40 μ in length, with diameter varying from 1.4 to 2.1 μ. On germination there is first an increase in size and the septa become more clearly visible because of the constrictions (fig. 9, B). The first germ tubes usually arise at the ends of the conidia, after which one or two hyphae arise from each of the cells. The mycelium is delicate at first, but in mature colonies it is composed of densely branched, interwoven hyphae the cells of which are brown, thick-walled, and beadlike (fig. 9, C).

INOCULATION EXPERIMENTS

The brown-spot fungus appears never to have been proved, by inoculation trials, to be parasitic. The writers attempted to prove its pathogenicity by use of suspensions of conidia from pure cultures. These suspensions were atomized, in late afternoon, upon healthy plants which had just begun to set seed. Plants growing in beds in the greenhouse were employed in some of the trials, and plants growing out of doors were used in others. When it was found that infections developed so promptly no attempt was made to conserve high degrees of humidity around inoculated plants, and only a few trials were made. In all cases by

the fifth day small, brown discolorations were present in abundance. Characteristic brown-spot lesions had developed a week later, while nearby plants which served as controls remained free from disease. The organism was reisolated from certain of these artificially inoculated plants by use of tissue plantings.

The method of entrance of the fungus was determined by sowing conidia in drops of water on leaves. Then after 16 to 20 hours, by examination in surface view of the epidermis stripped from these leaves, it was observed that the germ tubes enter the stomates (fig. 9, A). As growth continues the hyphae become much branched and ramify between the cells of the leaf tissues.

IDENTITY

The fungus which causes brown spot was compared by Hemmi [6] with *Septoria sojina* Thüm., the only other species known to be parasitic on soy bean, and was found to differ in several essential respects.

FIG. 7.—Soy-bean pods attacked by the brown-spot fungus

He therefore described it as a new species, *Septoria glycines*. The similarity between the pycnidial and conidial measurements of this fungus and those of the fungus with which the writers have been dealing led to the opinion that they were identical. Fortunately it was possible to establish this identity by comparison with Hemmi's type material, collected July 19, 1914, at Tsukisappu, Ishikari, which was sent to the writers. Specimens collected at Raleigh, N. C., were in turn sent to Hemmi for identification. He stated: "I am of the opinion that the fungus on your specimen is macroscopically as well as microscopically the same as my species, *Septoris glycines* Hemmi." [7]

[6] HEMMI, T. A NEW BROWN-SPOT DISEASE OF THE LEAF OF GLYCINE HISPIDA MAXIM. CAUSED BY SEPTORIA GLYCINES SP. N. Sapporo Nat. Hist. Soc. Trans. 6 : 12–17. 1915.

[7] From a letter dated Mar. 15, 1924, from Takewo Hemmi, Kyo o Imperial University, Kyoto, Japan.

OVERWINTERING AND DISSEMINATION

Diseased leaves and stems from the crop of 1922 and that of 1923 were collected and stored out of doors in unsuccessful attempts to obtain an ascogenous stage. The conidia in such material have been found to survive the winter and be still viable, as shown by germination tests made during March. Refuse from a diseased crop of the preceding year could therefore be expected to serve as a source of inoculum when the same field is returned to soy beans.

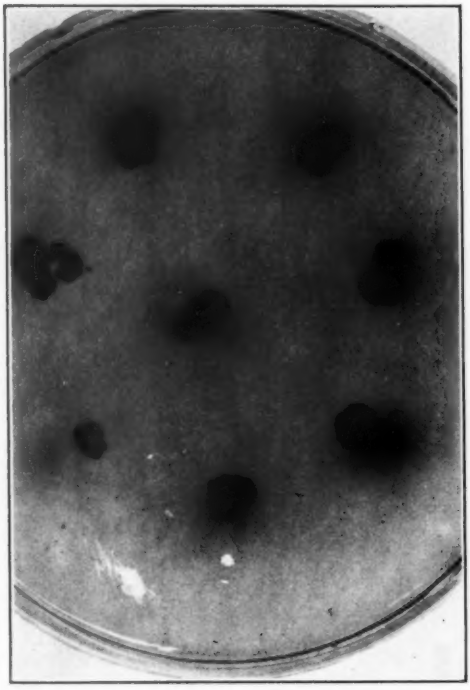

FIG. 8.—Three-weeks-old colonies of *Septoria glycines* on potato agar

The fact that the pods are subject to attack suggests the probability that brown spot is seed-borne and that contaminated seed are the agency by which it is disseminated. To date, the writers have not been able to demonstrate by cultures and by the aid of a microscope the presence of the fungus within the tissues of the seed or of the conidia on the surface of the seed coats. It has been pointed out, however, that the cotyledons bear lesions. This evidence of seed-borne infection is supplemented by the occurrence of brown spot in a small planting in 1924 from seed from a severely diseased crop in 1923. This planting was made on freshly cleared land, and was well isolated from other soybean fields. The presence of brown spot in this field appears to be most reasonably accounted for by its introduction with the seed.

VARIETAL RESISTANCE

The observations on this phase of the problem are very meager and cover only the year 1923, when brown spot was epiphytotic. Just as is the case with other diseases of this crop there are very manifest differences in varietal resistance. Among the varieties grown in the experimental breeding plots which were noted to be severely affected are Black Eyebrow, Virginia No. 12, and a considerable number of hybrids of Virginia. Austin, Wilson Black, Midwest,

and Ito San exhibited only a moderate degree of infection. Mammoth Yellow and Haberlandt 38, the two most commonly grown varieties in North Carolina, were only slightly affected. In this last group belong also Laredo, Biloxi, Lexington, Tokyo, Tar Heel Black, and Chiquita.

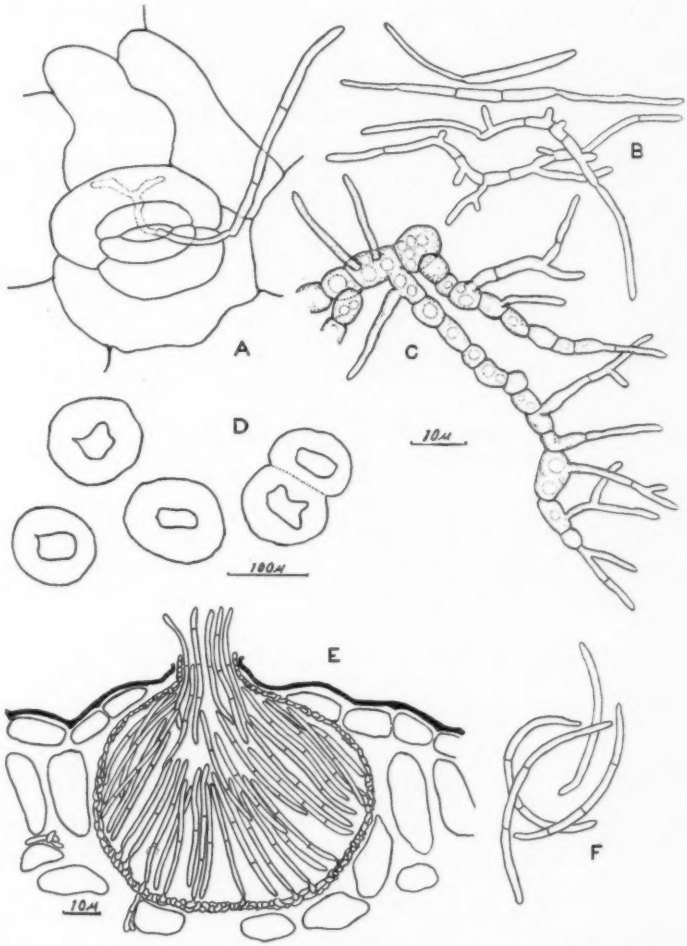

FIG. 9.—A, germination of conidium of *Septoria glycines*, and entrance of the germ tube into the leaf through a stoma; B, germination of conidia of *S. glycines*; C, hyphae from old cultures, with beadlike thick-walled cells; D, surface view of pycnidia of *S. glycines*, showing large ostiola; E, pycnidium in section which opens to the upper leaf surface; F, conidia of *S. glycines*. (A, B, C, and F are drawn to the same scale)

SUMMARY

This investigation deals with a disease of soy beans commonly called "brown spot," first described in Japan in 1915, where it was

collected in several Provinces. It occurs also in Manchuria and Korea, and was first noted in North Carolina in 1922 and in Delaware in 1923.

Brown spot manifests itself by the presence of angular brown or reddish-brown lesions on the foliage. It attacks also the stems and pods.

The disease seems to be seed-borne. It appears first on the cotyledons, spreads thence to the unifoliate leaves, from these to the trifoliate leaves, and causes defoliation from below upward.

Pathogenicity has been established by artificial inoculation. Infection occurs through the stomates, and the mycelium is intercellular.

Brown spot is caused by the imperfect fungus, *Septoria glycines* Hemmi. No ascogenous stage is known. The conidia remain viable during winter on decaying stems and leaves.

PYTHIUM ROOT ROT OF SOY BEAN[1]

By S. G. LEHMAN, *Plant Pathologist, North Carolina Agricultural Experiment Station*, and FREDERICK A. WOLF, *Plant Pathologist, Office of Fruit Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

So far as the writers are aware, the fungus *Pythium debaryanum* Hesse has never been reported as a parasite of soy bean. During a protracted rainy period in July, 1923, soy-bean plants in the breeding plots at the North Carolina Agricultural Experiment Station were found to be affected by a root-rot disease which was manifestly different from wilt (*Fusarium tracheiphilum* Smith) and sclerotial blight (*Sclerotium Rolfsii* Sacc.), the two root-rot diseases most commonly found on the soy bean in North Carolina. Tissue plantings on acidulated-agar plates were made from root lesions of these diseased plants, and the resultant cultures were identified as those of a Pythium of the *debaryanum* type. The results of a study of the morphology of this organism and of its relationship to the disease are recorded in this paper.

Nothing is known of the distribution of this root-rot disease, but since the fungus is cosmopolitan it is to be expected that, under suitable conditions, the disease may occur wherever soy beans are grown. It might appear as a root rot of large plants, as in the case in which it was first noted, and also as the cause of a damping off of seedlings.

THE DISEASE

Attention was first attracted to the disease by the presence of groups of withered and dying plants scattered irregularly throughout the field. The roots and basal portions of the stems of these affected plants were decaying (fig. 1). The lesions normally extended entirely around the stems. Apparently entrance had been effected at the ground level, whence the fungus spread downward and invaded the root system and at the same time extended upward several inches above the ground level, in many instances reaching the lowermost branches.

It has been found that the recently invaded stem tissues are translucent, whereas the older portions of the lesions are brown, necrotic, and involved in a wet rot. In old lesions the less resistant parenchyma is completely disintegrated, but the tougher, fibrous portions remain, the diseased areas assuming a dry, shredded appearance.

When plants are removed from the soil the cortical tissues of the larger roots are found to be so badly disintegrated that they fall away and leave the central woody cylinder exposed. The smaller roots are either completely decayed or break away when the plants are pulled.

[1] Received for publication Jan. 9, 1926; issued August, 1926.

THE FUNGUS

MYCELIUM

The fungus which causes this disease is easily isolated, and it grows well on a variety of media. Potato agar is especially favorable for mycelial development. The tips of young hyphae vary in diameter from 3.5 to 4.5 μ, and are taper pointed. They are finely granular in appearance, but as the hyphæ become older the granules enlarge and appear to collect in small clumps. The older hyphae are quite variable in thickness, ranging from 4 to 8 μ.

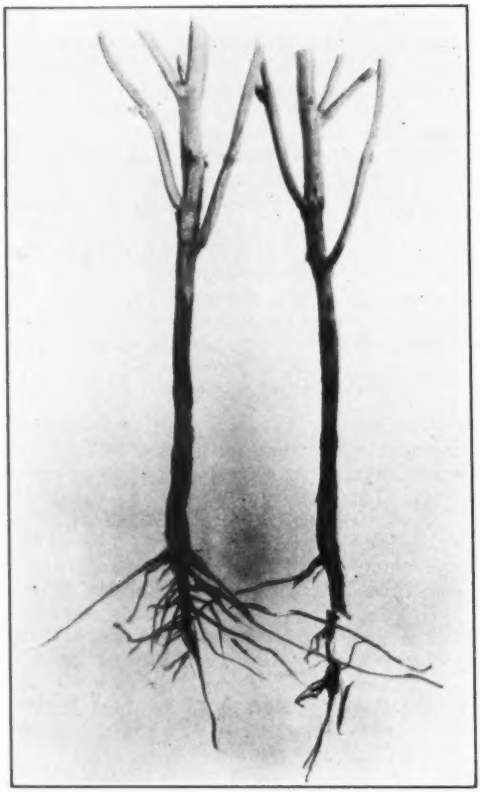

FIG. 1.—Root rot of soy bean caused by *Pythium debaryanum*

OOGONIA AND OOSPORES

Two types of reproductive bodies, conidia and oogonia, with their accompanying antheridia, have been observed. True sporangia have not been noted. Oogonia and antheridia are formed sparsely or not at all on potato-dextrose agar. However, if bits of diseased soy-bean tissue are placed in water in a Petri dish and kept at a favorable temperature, oogonia and antheridia are formed in great numbers in three to five days. On this substratum the oogonia are spherical, colorless, smooth, and have a diameter ranging from 16 to 24 μ, with an average of 20.5 μ. In cultures on insects the oogonial dimensions are somewhat greater, ranging from 20.7 to 32.72 μ, with an average of 23.8 μ. Mature oospores grown on vegetable tissue have an average diameter of 17.2 μ; they are smooth, light brown, thick-walled, and vary in diameter from 12 to 18.5 μ. The protoplasm of the oogonium is at first homogeneous and finely granular.

Initiation of development of the oosphere is marked by a more or less conspicuous clumping of the protoplasmic granules and contraction of the ooplasmic material, leaving a clear periplasmic peripheral zone between the wall of the oogonium and the oosphere. After fertilization, a thin, clear, membrane or vesicle appears about the oosphere, and it thickens and becomes the oospore wall.

ANTHERIDIA

Two types of antheridia, stalk and branch, are developed. In the production of a stalk antheridium, that portion of the oogoniophore immediately subjacent to the oogonium is delimited by the formation of a cross wall. This cell functions as an antheridium and develops a fertilization tube through which the antheridial contents pass into the oosphere (fig. 2, U, V). Branch antheridia arise either as branches of the oogoniophore or of a neighboring hypha. In cases in which the antheridial branch arises from the oogoniophore, the antheridium itself may be supported by a stalk cell or it may be attached directly to the oogoniophore. The branch antheridium is a club-shaped, cylindrical, usually curved cell whose diameter is usually 1.5 to 2.5 times that of its stalk or supporting hypha and whose length is 3 to 5 times its diameter (fig. 2, S). Branch antheridia are not broadly or closely applied to the oogonium, and do not in any case envelop it, but they usually curve in such a way that only their tips are in contact with the oogonium. The protoplasm of both oogonia and antheridia is at first finely granular. When the antheridium comes in contact with the oogonium, the granules in both parts enlarge and collect in clusters, and, in the oogonium, withdraw from the wall leaving a clear margin of periplasm (fig. 2, T). The fertilization tube pushes through the oogonial wall and can be clearly seen to penetrate to the contracted ooplasm. It may have a diameter as great as 2.5 μ. The fertilization tube from the stalk antheridium pushes directly through the septum which separates the antheridium from the oogonium and may easily be observed in situ. Fertilization tubes persist for a considerable time after the formation of the oospore wall. The antheridia persist also after fertilization, but they appear to be empty except for a few scattered granules (fig. 2, R and W).

CONIDIA

Cultures on soy-bean stems in water produced numerous globular bodies which were at first thought to be sporangia. Numerous efforts to induce germination, however, resulted either in the conidial type of germination or no germination at all. Conidia are of two kinds, either terminal or intercalary. Those formed terminally are spherical, while those formed intercalarily have slight polar projections at the positions of the septa which delimit them from their supporting hyphae. They have thin hyaline walls, and are densely filled with protoplasm which contains numerous clusters of fine granules (fig. 2, A to J, inclusive). In size, terminal conidia are approximately equal to ripe oogonia. They will not germinate in the stale water of the cultures in which they have been formed, but germination may become evident within 30 minutes after the conidia are placed in fresh tap water at a suitable temperature (fig. 2, M to Q, inclusive). They germinate well under a cover glass on a slide provided a continuous supply of fresh water is made to flow slowly through the preparation by means of a wick. The germ tube grows rapidly and is filled with clusters of finely granular protoplasm except at the tip where it is homogeneous and clear. As the germ tube elongates, the contents of the spore passes into it and leaves the spore apparently empty. As many as three germ tubes may arise from one conidium.

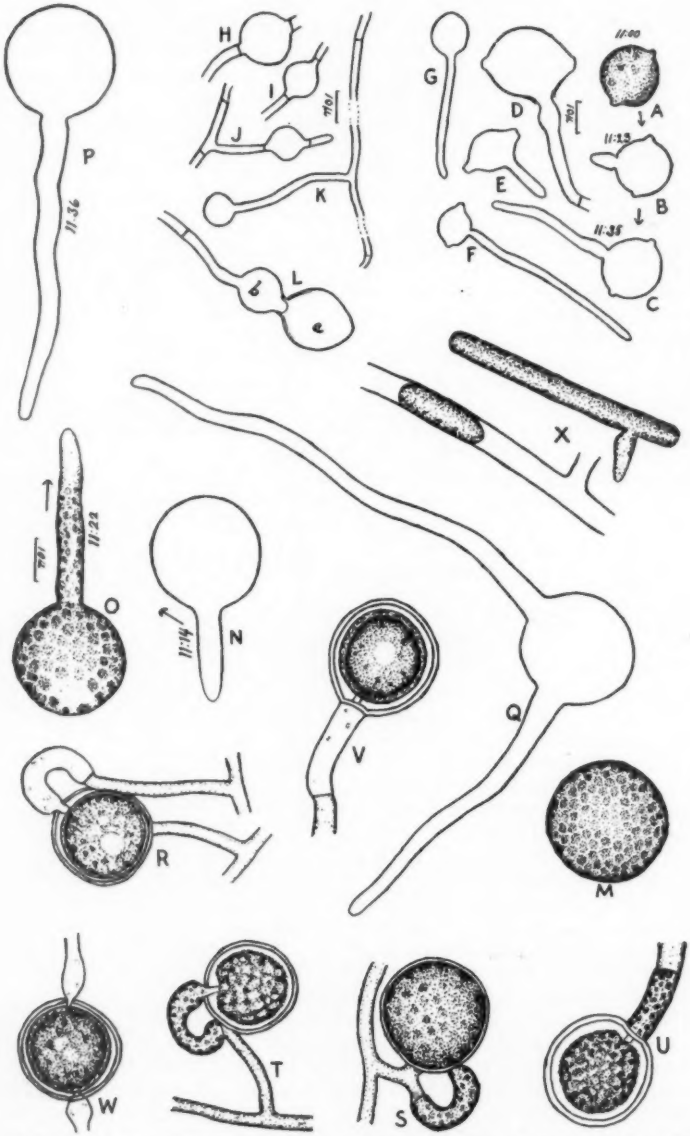

FIG. 2.—The development of *Pythium debaryanum*, the causative fungus in the root rot of soy bean

(For explanatory legend, see p. 379.)

Secondary conidia have also appeared in the cultures (fig. 2, L). Fragments of old hyphae, in cultures a few months old, when placed in fresh water may germinate after the manner of primary or secondary conidia and thus function as propagative bodies (fig. 2, X).

INFECTION EXPERIMENTS

Soy beans about a month old, which were growing in a bed in the greenhouse, were used in the inoculation trials. Cultures were macerated in sterile water and poured on the surface of the ground near the plants. The plants were then shaded with bell jars for 24 hours. Seven days later lesions were present on all of the plants in the area to which the inoculum had been applied. In those cases in which wilting and drooping had begun to be manifest, the cortex was beginning to disintegrate near the ground level in a characteristic manner. In the course of another week a few of the inoculated plants had succumbed, and others bore well-defined lesions on both stems and roots. Plants in the same bed but distant from those in the area to which the inoculum was applied served as controls; and these continued to remain healthy.

The causal organism was reisolated from certain of these artificially inoculated plants and found to be identical with the one used in the inoculation experiments.

TAXONOMY

The character of the mycelium of the soy-bean fungus, the type of conidia, the method of their formation, and the morphology of the sexual organs are clearly those of the genus Pythium. The absence of filamentous sporangia and the presence of conidia refer it to the subgenus Sphaerosporangium of Butler's [2] classification, and the smooth oogonia and oospores place it in a group of seven species of which *Pythium debaryanum* is one. In this group *Pythium rostratum*, *P. vexans*, and *P. ultimum* are known to be saprophytes. *P. palmivorum* has pear-shaped sporangia and does not bear conidia, and *P. anguillulae aceti* and *P. intermedium* have catenulate conidia and sporangia. The morphological characters of the soy-bean fungus

[2] BUTLER, E. J. AN ACCOUNT OF THE GENUS PYTHIUM AND SOME CHYTRIDIACEAE. India Dept. Agr. Mem., Bot. Ser., v. 1, no. 5, 160 p., illus. 1907.

EXPLANATORY LEGEND FOR FIGURE 2

A, B, C, D, E, F, G.—Intercalary conidia germinating in tap water. The rapidity of germination and growth of the germ tube is shown by the time given immediately above A, B, and C.

H, I, J.—Intercalary conidia with supporting hyphae.

K.—A developing conidium, which may become terminal or intercalary.

L.—Conidium (*a*) that has germinated and formed a secondary conidium (*b*).

M.—Conidium formed terminally, showing character of its contents.

N, O, P, Q.—Conidia formed terminally, germinating in a continuous stream of water under a cover glass. The rapidity of growth of the germ tube is indicated by the time given alongside N. O, and P.

R.—Oogonium and branch antheridium arising from different hyphae. Fertilization has occurred, as is indicated by empty antheridium and the thin wall about the oospore.

S.—Oogonium with branch antheridium arising from the oogoniophore. No fertilization tube yet evident, nor has the oosphere begun to withdraw from the wall of the oogonium.

T.—Oogonium with branch antheridium arising from the oogoniophore. Oosphere contracted, and fertilization tube in contact with it. Contents of the antheridium have not yet passed into the oosphere.

U, V.—Oogonia with stalk antheridia. Fertilization had occurred in V. The oospore has a thick wall, and finely granular protoplasm with a large oil drop.

W.—Oogonium with two antheridia.

X.—Portions of hyphae filled with dense, finely granular protoplasm. These are resting cells which may serve as propagative structures. The upper one is germinating.

(All figures were drawn with the aid of camera lucida. The magnification of A to L, inclusive, is indicated by the scale shown alongside D and K; that of M to X, inclusive, by the scale shown alongside O.)

make it impossible to regard it as cospecific with either of these six species. In view of its close similarity to *Pythium debaryanum* it is referred to that species. No sporangia such as have been described for this species by de Bary and Butler have been observed by the writers, but instead they have observed numerous conidia which may perhaps be regarded as potential sporangia. This failure to form sporangia and the abundant production of conidia appears to be of common occurrence in strains of this fungus which are referred by various investigators to the species *debaryanum*.

SUMMARY

Soy beans are subject to a root-rot disease which was first observed to be present in North Carolina in 1923.

This malady manifests itself by the presence of necrotic wet-rot lesions, which involve the stems and roots near the ground level and cause the plants to wither and die.

The causal organism accords well in mycelial characters and morphology of conidia, oogonia, and antheridia with *Pythium debaryanum* Hesse, with which it is believed to be identical.

SOY-BEAN ANTHRACNOSE[1]

By S. G. LEHMAN, *Plant Pathologist, North Carolina Agricultural Experiment Station*, and FREDERICK A. WOLF, *Plant Pathologist, Office of Fruit Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

An anthracnose disease of soy bean, *Soja max* (L.) Piper, was found to be present at the North Carolina Agricultural Experiment Station in 1920, in the plots devoted to investigation of this crop. A brief statement was prepared at that time (*7, p. 57*)[2] which is believed by the writers to have recorded for the first time the occurrence of this disease within the United States, and tentatively assigned to the causal organism the name *Glomerella cingulata* (Stonem.) V. Sch. and S. Since then the disease has appeared at the station in succeeding years and has been found in a number of other localities in the State, which has afforded opportunity for a more intensive study of it. It is the present purpose to describe the disease and to record the results of investigations into the morphology and life history of the causal organism.

HISTORICAL

A survey of available literature on soy-bean diseases shows that anthracnose has been noted only in the Orient. Hemmi (*2, 3*) records the isolation by S. Takimoto of an unnamed species of Gloeosporium from diseased soy-bean pods collected in Suigen, Chosen (Korea), in October, 1915. No inoculation experiments were made with this organism, so proof of its parasitism is wanting.

The same investigator isolated in September, 1917, another anthracnose fungus, which was sent to Hemmi (*2, 3*), and which he identified as *Colletotrichum glycines* Hori. Hori has not published a technical description of this species,[3] although its important morphological features, accompanied by appropriate drawings, are given in a report of investigations by Hemmi (*2*). Neither has this anthracnose fungus been employed in inoculation experiments, but its prevalence on stems and pods at harvest time, together with a knowledge of other species of this genus, was taken by Hemmi to indicate that it was actively parasitic.

DESCRIPTION OF SOY-BEAN ANTHRACNOSE

Soy-bean anthracnose has been observed on the stems and pods, but apparently it does not affect the foliage. Plants in all stages of growth are subject to infection, as shown by field observation and

[1] Received for publication Jan. 9, 1926; issued August, 1926.
[2] Reference is made by number (italic) to "Literature cited," p. 389.
[3] This statement is taken from a letter from Dr. Takewo Hemmi, Kyoto Imperial University, Kyoto, Japan, dated Aug. 24, 1924.

inoculation experiments. The disease reaches its most destructive stage of development in late summer, when the pods are maturing, especially during rainy periods. At this time no definite lesions such as characterize other anthracnose diseases are present, but the affected portions are thickly covered with setose, black acervuli (fig. 1). The macroscopic appearance somewhat resembles the pod and stem blight, *Diaporthe sojae* Lehm., but the fruit bodies of the two may be distinguished with certainty by the use of a hand lens. Moreover, the acervuli are irregularly disposed at the surface of affected stems, with no tendency to arrangement in lines as occurs with the Phomopsis stage of the pod and stem blight fungus. Affected plants which are scattered irregularly throughout the field may be readily recognized because of their premature death.

The first evidence of disease is the presence of minute pinpoint-like dark discolorations at the loci of infections, which are visible under favorable conditions four days after inoculation. The surrounding tissues become brown and dead, and by the fusion of individual lesions large areas are formed which may involve the entire surface of the stems and pods. If the pod and pedicel are attacked early enough, seed formation may be partly or entirely inhibited. This may be accomplished by the invasion of the tissues of the pedicel, and in consequence the pods fail to fill, or the mycelium may penetrate the pod wall directly and attack the developing seed. Shrunken seed from such pods invariably yield pure cultures of the anthracnose organism when such seeds are subjected to surface disinfection and plantings are made from seed-coat or cotyledonary tissue.

There is little or no shrinking and collapse of stems because of the large proportion of woody tissue and small proportion of parenchyma.

CAUSAL ORGANISM

ISOLATION

Isolations have been made from diseased stems and pods bearing the conidial stage, from infected seed, and from old, decaying stems bearing the ascogenous stage. Suspensions of conidia in sterile water have been employed in plantings in hardened-agar plates, and this has been found to be a satisfactory method of isolation. Stems bearing the Glomerella stage have been placed on bibitory paper in the tops of inverted Petri dishes containing hardened agar. The ascospores are forcibly ejected, lodge on the surface of the agar, and yield pure cultures of the organism by this method. No efforts have been made to make single-spore cultures from either conidia or ascospores, but blocks of agar containing several spores have been transferred to culture tubes. The cultures from all sources, however, have yielded the same type of growth. The mycelium is whitish and rather loose at first, but soon turns dark and the substratum becomes blackened. Within two or three weeks black acervuli bearing pink spore masses and black perithecia have formed. Some strains from conidia have borne conidia alone, even after repeated transfer and cultivation on a variety of media; other strains from ascospores, however, have consistently yielded both the conidial and ascosporic stages when cultivated upon the same kind of media.

MORPHOLOGY

As the soy-bean anthracnose fungus was at first thought to be identical with *Glomerella cingulata*, it was grown in parallel cultures with the apple bitter-rot fungus isolated from lesions on apple fruits. The decaying fruits from which these isolations were made

FIG. 1.—Soy-bean anthracnose on pods and stems, showing the distribution of black acervuli

bore at the same time both the conidial and ascogenous stages of the bitter-rot fungus. These in turn fruited in culture with the formation of both stages, so that it was possible to make comparative measurements of the organism from apples and the one from soy beans both from natural sources and from artificial cultures. Table 1 contains a summary of the relative length of ascospores of both organisms from cultures.

TABLE 1.—*Relative length of ascospores of the soy-bean anthracnose fungus and the apple bitter-rot organism*

[One space=3.75 μ]

Organisms	Number of spores distributed according to length in spaces																Variation in length (μ)	Common length (μ)
	Spaces																	
	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0		
Soy-bean anthracnose fungus	0	0	6	27	8	74	23	70	30	17	14	3	3	1	1	2	13.12-43.35	18.75-28.12
Apple bitter-rot fungus	16	62	45	61	24	16	0	0	0	0	0	0	0	0	0	0	9.37-18.75	11.25-15.00

Comparing the ascospores of the two organisms grown in parallel cultures, it will be noted that those of the soy-bean organism are considerably larger than those of the apple organism, the former varying in length from 13.12 to 43.35 μ, and the latter from 9.37 to 18.75 μ. These differences are more apparent when it is observed that 75 per cent of the ascospores of the bitter-rot fungus range from 3 to 4 spaces, or 11.25 to 15.00 μ in length, whereas 76.6 per cent of those of the soy-bean anthracnose range from 5 to 7 spaces, or 18.75 to 26.25 μ. They range in width from 3.9 to 5.0 μ, and 4 to 6 μ, respectively.

When ascospores are taken directly from decaying host tissue they appear to be more uniform in size, i. e., there are fewer either longer or shorter than the average, than on artificial media. The average measurements accord, however, without regard to the substratum upon which they are grown, and agree well with measurements of the bitter-rot fungus given by Clinton (*1*) and by Von Schrenk and Spaulding (*4*). Their extremes range from 12 to 22 by 3.5 to 5 μ.

The asci of the soy-bean fungus vary from 70 to 106 μ in length and from 9.5 to 13.5 μ in width, with an average length of 80 μ and an average width of 12 μ. The asci of the apple bitter-rot fungus vary from 55 to 70 μ in length and from 9 to 10 μ in width, with an average length of 60 μ and an average width of 9 μ. The perithecia of the anthracnose fungus vary from 220 to 340 μ in diameter. The perithecia of the apple bitter-rot fungus vary from 125 to 250 μ in diameter, which measurements accord with those given by Clinton (*1*) and by Von Schrenk and Spaulding (*4*).

The Colletotrichum stage of soy-bean anthracnose bears conidia which vary in length from 16 to 25 μ, but the most common size is 20 to 22 μ long by 3.75 to 4.5 μ wide. There are numerous brown setae which vary from 30 to 200 μ in length and from 4 to 6 μ in width. It agrees well with Hemmi's (*2*) description of *C. glycines*, in which the conidial size is given as 16 to 23 by 3.8 to 4.2 μ, with 15 to 40 setae in each acervulus.

The Gloeosporium stage of the bitter-rot fungus is given by Clinton (*1*) and by Von Schrenk and Spaulding (*4*) as having extreme lengths of 10 to 28 μ and extreme widths of 3.5 to 7 μ, with 12 to 16 by 4 to 5 μ as a common size. These measurements agree well with those made by the writers. Measurements of 440 conidia, half of

which were taken from culture on potato-dextrose agar and the other half from a decaying apple, showed an extreme range in length of 6 to 22 μ with 11 to 16 μ as the most common. The conidia grown in culture exhibited a wider range and averaged slightly smaller than those grown on the fruit..

The cultures of soy-bean anthracnose and apple bitter rot exhibit readily distinguishable differences, as shown in Figure 2. Colonies of the former are whitish at first, but soon become smoky black with darkening of the substratum; colonies of the bitter rot are also whitish at first, but become darker (olive tinted) only around the perithecial stromata and acervuli.

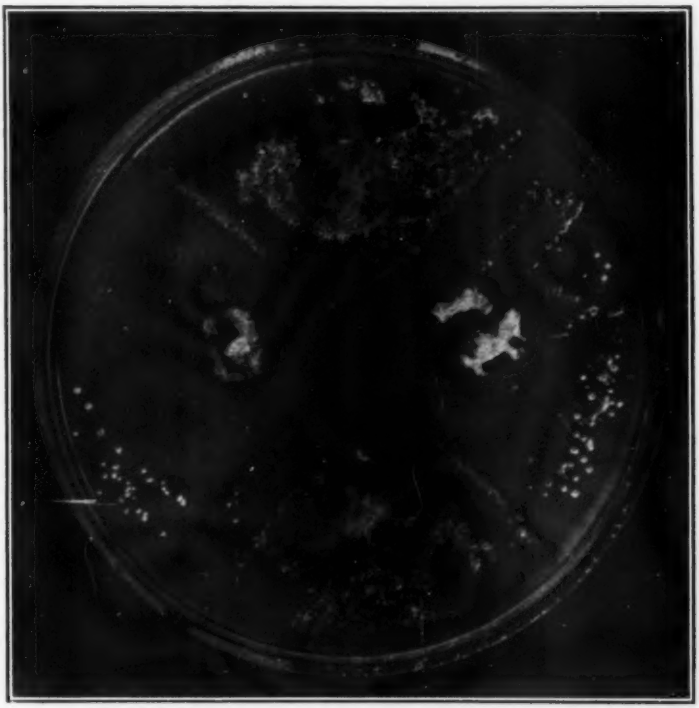

FIG. 2.—The soy-bean anthracnose and apple bitter-rot organism in planted agar plate. The upper and lower quadrants show the bitter-rot fungus, and the right and left quadrants the soy-bean fungus

INOCULATIONS

The inoculation experiments had for their object the determination (1) of pathogenicity of the anthracnose fungus to soy beans, which had not been previously demonstrated, and (2) of the relationship between anthracnose of soy bean and bitter rot of apple. Soy-bean plants, in several series of trials, were inoculated by atomizing them with suspensions of conidia and ascospores from

pure cultures. Certain of the plants were inoculated with the soy-bean fungus, and others with the bitter-rot organism, under identical conditions. Inoculations were made in late afternoon on plants growing in the greenhouse, and the inoculated plants were shaded with newspapers for 24 hours. Each of the series gave uniformly the same results. Within three to four days the first evidences of infection were noted on plants inoculated with the soy-bean organism. Those which were inoculated with apple bitter rot failed to develop any evidence of infection during the entire period in which they were under observation. In another trial three soy-bean plants were inoculated by inserting bits of mycelium and spores into the stems, but here again no infection resulted and the wounds healed promptly.

Reciprocal inoculations were also attempted on several varieties of apples in the following manner: After surface disinfection the fruits were placed in moist chambers and inoculated, on July 2, on opposite sides of the apple. The inoculum, consisting of mycelium and spores from pure culture, was inserted in needle punctures. The tissues surrounding the points of inoculation became involved in decay in the case of all punctures with both organisms. By July 15 the characteristic sunken areas with rings of acervuli had formed in the case of inoculations with bitter rot, whereas the tissues were somewhat darker and less soft in those inoculated with anthracnose of soy bean, and no acervuli had formed on the surface. Furthermore, the characteristic bitter taste was lacking in tissues decayed by the soy-bean fungus.

LIFE HISTORY OF THE FUNGUS

The fungus which causes soy-bean anthracnose has a Colletotrichum or conidial stage and a Glomerella or ascosporic stage. The germination of both types of spores is essentially alike. As an initial step in this process a median septum is generally formed, although many spores remain unicellular. This is followed by the formation of a short germ tube, which is terminated by a brownish appressorium from which the infection tube later arises (fig. 3, F, H). In the inoculation trials in which spores were placed in drops of water on young pods, entrance had been effected within 48 hours by direct penetration of the cell wall (fig. 3, C). The mycelium rapidly extends to adjoining cells and causes their death. It is both inter and intra cellular. Acervuli mature on the lesions in 10 to 14 days.

The fungus passes the winter season either by means of infected seed or of the ascogenous stage. Its occurrence within the tissues of the seed has been established by isolation after surface disinfection, from seed taken from diseased pods. Indirect evidence that anthracnose is seed-borne was obtained by planting seed from affected pods in a situation in which soy beans were not known ever to have been grown, and which was at least half a mile from the nearest soy-bean field. All of the plants in the resultant crop from such seed were seriously affected. In the light of knowledge of other anthracnose diseases it seems reasonable to suppose that the spores which find lodgment on the surface of the seed during harvest could

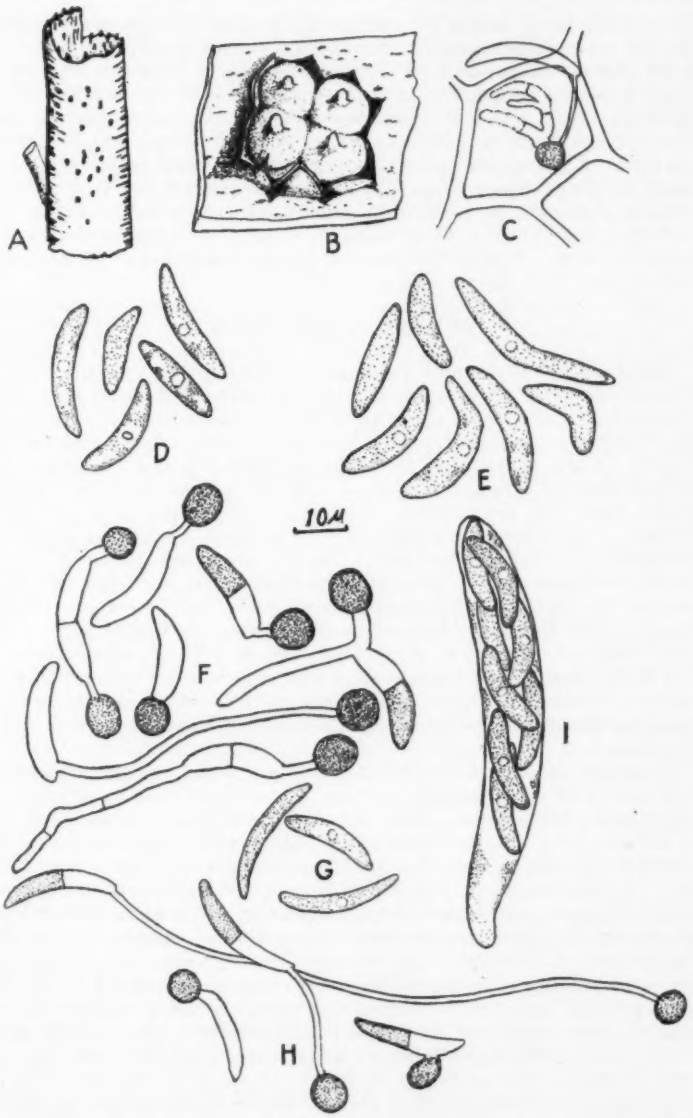

FIG. 3.—A, Diagram of soy-bean stem showing disposition of perithecial stomata; B, diagram of a single stroma bearing four innate perithecia of *Glomerella glycines;* C, germination of conidium and penetration of pod wall by infection tube 48 hours after inoculation, surface view; D, ascospores of *Glomerella glycines* from decaying soy-bean stems; E, ascospores from artificial cultures; F, types of germination of ascospores after 16 hours in water; G, conidia from the Colletotrichum stage; H, germination of conidia with resultant appressoria; I, ascus of *Glomerella glycines*

also serve as a means of carrying the organism over the winter period and of disseminating it in new fields or localities.

In March, 1924, stems of diseased soy beans from the crop of the previous year which had remained in the field during the winter were found to bear the Glomerella stage. The stromata in which the perithecia are embedded arise within the cortex and are at first covered. Each stroma gives rise to one or several perithecia, whose short beaks protrude (fig. 3, A, B). The ascospores are ejected as the asci mature, as previously shown by the isolation trials with inverted agar plates. It is very probable that some of these would come to lodge on plants of the next crop if soy beans were planted in the same field the succeeding year.

IDENTITY OF THE FUNGUS

Plant pathologists would place one or the other of two interpretations upon the foreging studies on the morphologic and pathogenic characters of the soy-bean anthracnose fungus. Some would regard this fungus as specifically identical with *Glomerella cingulata* from apples. Such an opinion is indicated from Shear's (*5*) studies on anthracnose of a large number of different plants, in which he concludes that "all are perhaps only slightly specialized physiological forms of one omnivorous species." The same idea is expressed by Taubenhaus (*6*) from his studies on Gloeosporium on sweet pea, in which he states (1) "that the anthracnose disease of the sweet pea is due to the same organism, *Glomerella rufomaculans* (Berk.) Spauld. and Von Sch., that causes the bitter rot of the apple," and (2) "that *Gloeosporium officinale* E. and E., *Gloeosporium gallarum* Ch. Rich., and *Gloeosporium sp.* from May-apple fruit are also the same as *Glomerella rufomaculans*, since they are all able to produce the typical anthracnose disease of the sweet pea and the bitter rot of the pear."

There are others, among whom are the present writers, who would conclude that soy-bean anthracnose is specifically distinct from the apple bitter-rot fungus. The reasons for this may be summarized as follows: (1) The perithecia, asci, ascospores, and conidia of the soy-bean fungus are larger than those of the apple bitter-rot organism; (2) the conidial stage of the former belongs to the form genus Colletotrichum, the latter to Gloeosporium; (3) when the two are grown on the same substratum the colonies present readily distinguishable differences; (4) the soy-bean organism is pathogenic to soy beans whereas the fungus from decaying apples failed to produce infections on soy beans; (5) the type of decay produced by the fungus from soy beans on apple fruits possesses none of the characteristics associated with apple bitter rot. The fact that the soy-bean fungus causes a decay of apple fruits need not be taken to prove its parasitism, since a mature apple is essentially a culture medium. As is well known, apples can be rotted by a considerable number of fungi not regarded as parasites. More significance certainly should be attached to the fact that the bitter-rot fungus will not attack soy beans than that the anthracnose fungus will produce a rot of apples.

Since it has thus far been impossible to make comparison with specimens from Hori's original collections or with any other collections of *Colletotrichum glycines* from the Orient, the writer does not know with certainty whether the form with which they have been working is identical with *C. glycines*. The descriptive characters mentioned by Hemmi (*2*) accord sufficiently well, however, to lead to the belief that they are one and the same species. Since the perfect stage is herein appropriately described for the first time, and its relationship to *Colletotrichum glycines* established, a brief technical summary is given, as follows:

Glomerella glycines (Hori.) n. n.

Syn. *Colletotrichum glycines* Hori.

PERITHECIAL STAGE.—Perithecia membranaceous, rostrate, caespitose, 220 to 340 μ in diameter, immersed in a stroma. Asci oblong to bluntly clavate, a paraphysate, 70 to 106 by 9.5 to 13.5 μ; ascospores hyaline, slightly curved, blunt-pointed, unicellular, 13.12 to 43.35 μ in length, chiefly 18.75 to 28.12 μ, by 4 to 6 μ in width.

Hab.: On decaying stems of *Soja max* (L.) Piper.

CONIDIAL STAGE.—Lesions on stems and pods indefinite in outline. Acervuli black, setae numerous, brown, 100 to 200 μ in length, Conidia hyaline, curved, bluntly tapered, unicellular, 16 to 25 $\times$ 3.75 to 4.5 μ, with 20 to 22 μ as the most common length.

Hab.: Parasitic on stems and pods of *Soja max* (L.) Piper.

SUMMARY

Soy-bean anthracnose affects the stems and pods of this crop and its presence in North Carolina was first observed in 1920. It is believed to be identical with a disease collected in Chosen in 1917 and ascribed to *Colletotrichum glycines* Hori.

The disease is characterized by the presence of numerous black acervuli, uniformly scattered over the surface of the affected parts. It causes premature death of the plants and failure of the pods to fill properly.

The organism is seed-borne, and exists as a mycelium within the seed and as spores adhering to the exterior.

When the soy-bean anthracnose fungus is compared with the fungus which causes apple bitter rot, with which it was at first believed to be identical. it is found to be morphologically distinct, to be of different appearance in culture, and to react differently in reciprocal inoculations.

The ascogenous stage has been found on diseased stems which overwintered in the field, and has been developed in culture. The name *Glomerella glycines* (Hori) n. n. is therefore proposed as a synonym for the conidial stage name *Colletotrichum glycines*.

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DISEASES OF SOY BEANS WHICH OCCUR BOTH IN NORTH CAROLINA AND THE ORIENT[1]

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INTRODUCTION

A number of the more important diseases of soy bean, *Soja max* (L.) Piper, have been investigated in the last 10 years at the North Carolina Agricultural Experiment Station (*3*, *4*, *9*, *10*, *11*, *12*, *15*, *17*, *18*, *19*).[2] Collections of diseased plants made in various parts of the State have been valuable in interpreting the detailed life-history studies of the causal organisms. The writers' information as to the distribution and prevalence of these diseases has been supplemented by specimens and letters of inquiry from county agents and growers. The most significant result of this work, which has arisen both from the laboratory studies and the field survey, has been the establishment of the fact that certain of these diseases are seed-borne. This fact accounts for the distribution of the diseases throughout the States in which soy beans are grown, serves as a basis for control, and suggests that some of the diseases probably were introduced into the United States with the seed. It is the primary purpose of this report to present such evidence as bears on the last phase of this problem and to give a brief account of the diseases which are common to the Orient and to North Carolina.

As is well known, the soy bean is native to eastern Asia and is a crop of great importance in Japan, Manchuria, Chosen (Korea), and Mongolia. It has been a major crop of the Orient since ancient times, but was of no importance as a farm crop in the United States until about 1880.

In the last 10 years there has been a progressive increase in soy-bean acreage in this country, especially in North Carolina and in several States of the Corn Belt. To-day soy beans are a major crop in the United States.

Apparently the fact that soy beans have only recently become of commercial importance in this country is responsible for the lack of knowledge of soy-bean diseases as found in the United States. A survey of available literature on soy-bean diseases in eastern Asia shows that a similar condition exists there, and that such information as has been recorded on soy-bean diseases in Asia has been contributed largely by Miura (*13*), Hemmi (*6*, *7*, *8*), Takimoto (*16*), and Nakata. The present writers' knowledge of soy-bean diseases in the Orient is limited by the fact that a considerable part of the work done there has been been published in Japanese. Collections of diseased plants and unpublished manuscripts and additional notes sent by Miura, Hemmi, and Nakata have been of invaluable service to the present writers in their work.

[1] Received for publication Jan. 9, 1926; issued August, 1926.
[2] Reference is made by number (italic) to "Literature cited," p. 395.

WILT

Soy-bean wilt was observed in North Carolina for the first time in 1915. It was ascribed to the same organism which causes cowpea wilt, *Fusarium tracheiphilum* Smith (*3*, *4*). This organism causes a browning or blackening of the xylem of roots and stems, and in advanced stages salmon-colored spore masses, sporodochia, are formed at the surface of the stems and lower branches. The organism is a soil-inhabiting fungus, which enters the plant through the roots. These characters it possesses in common with a considerable number of other Fusaria. This disease has been collected at various times in a number of localities in North Carolina, but does not seem to have been noted as involving the uppermost branches and seed pods.

During 1924, however, a serious Fusarium disease of the pods was noted in Japan. Specimens collected by T. Nojima at Kitashirakama, Kyoto, were sent to the writers by Hemmi, and the causal organism was found to be indistinguishable morphologically from *Fusarium tracheiphilum*. Another collection was made during the previous year near Kiyomitsu, Sizouka, by K. Hara, of apparently the same organism on diseased pods.

MILDEW

Mildew, when collected in North Carolina for the first time in 1923, was thought to be due to a hitherto undescribed species of Peronospora. When it was compared with the species which occur on legumes it was found to be most nearly like *Peronospora trifoliorum* de Bary. However, it differs sufficiently from this species in size of oogonia, of oospores, and of conidia to warrant regarding it as specifically distinct, and so the new name *P. sojae* was proposed (*10*). While the manuscript which recorded these findings was in press the writers' attention was directed to an account of this disease in Manchuria (*13*) in which the causal organism was identified as *P. trifoliorum* de Bary var. Manshurica Naoumoff (*14*). Further search revealed that in 1923 Gäumann (*5*) had raised this variety to specific rank and had accordingly appropriately employed for this mildew the combination P. manshurica (Naoumoff) Sydow in lit. When the writers' specimens were compared with collections made by K. Hara in Kanaya, Sizuoka, and by Hemmi in Higashi-Kutchan, Hokkaido, they were found to be identical and to agree morphologically with Gäumann's species *manshurica*. This name has priority, and so *P. sojae* must be regarded as a synonym.

Miura (*13*) has recorded the presence of mildew in Manchuria. Ocfemia and Weston stated in personal correspondence that they observed it in the Philippines. Butler (*1*) noted it in India.

Seed from a field in which mildew was abundant in 1923 was planted in an isolated situation the following season in order to obtain evidence of seed transmission of the malady. Mildew appeared on this planting late in June. At first only a few scattered lesions were noted on a few of the plants, but 10 days later the disease was very abundant on all of the plants and the lower surface of the lesions was covered with a violaceous coating of conidiophores. It is believed that in this case the organism was carried on the seed, and that seed-borne conidia served as the source of inoculum.

BROWN-SPOT DISEASE

This leaf-spot disease, which is due to *Septoria glycines* Hemmi, was recognized in North Cariolina for the first time in 1922. It was first collected in Japan in the province of Kitamai in 1914; it was epiphytotic that season in the provinces of Ishikari, Tokashi, and Iburi. In 1915 Hemmi (*6*) described the disease and its causal organism, and pointed out that the disease had been in Japan for years, and that it probably occurred throughout Asia wherever soy beans were grown. Hemmi sent to the writers specimens collected at Suwon, Chosen. Miura (*13*) noted it on soy beans throughout Manchuria, and he sent from Kung-Chu-Liug, South Manchuria, specimens of what appears to be the same organism on *Phaseolus munge* var. *radiatus*.

The disease in the Orient has been recorded as a leaf spot on both the primary and trifoliate leaves. It occurs in North Carolina also on stems, pods, and cotyledons, and has been shown to be disseminated by means of contaminated seed (*19*).

POD-AND-STEM BLIGHT

Pod-and-stem blight (*Diaporthe sojae* Lehm.) was found in North Carolina for the first time in 1920. No reports have come to the writers' attention of its occurrence subsequently in other States. However, in view of the fact that the disease appears on the pods, and the fact that the fungus either destroys the developing ovules or infects the seed, the disease undoubtedly occurs in other parts of the United States. There appears to be no mention of the presence of this disease in eastern Asia. The writers' knowledge of its occurrence there comes from a single collection made September 20, 1923, of the Phomopsis stage from Kiyomitsu, Shizuoka. These specimens were collected by H. Hara and sent by K. Nakata.

Lehman (*9*), in a comprehensive report of this disease, pointed out that the causal organism overwinters in the pycnidial stage on old diseased stems, and that the perithecial stage had never been found in the field but had been developed in culture. In March, 1924, however, the Diaporthe stage was found on stems which had overwintered in the field. Its relationship to the pycnidial stage was established by comparative measurements with the perithecial stage as had been developed in culture and with which it was found to agree, and by isolation from ascospores which when cultured gave rise to pycnidia like those of the Phomopsis stage.

CERCOSPORA LEAF SPOT

In 1918, Miura (*13*) collected at Tu-men-ling, South Manchuria, a leaf-spot disease which he ascribed to a new species, *Cercospora diazu Miura*. The spots are irregular to circular areas 3 to 6 millimeters in diameter, which are dark brown at first and become light brown with dark brown borders with age. Miura's brief mycological note appears to be the only published account of this disease, but it occurs in Japan, as Hemmi sent specimens collected at Nango, Miyagi, in 1924, and another collection from Shizuoka, Shizuoka, sent by Nakato appears to the writers to be that of this fungus. In September, 1925, one of the present writers (Lehman) found a leaf-spot disease near Moyock, N. C., which proved to be identical in size of

conidiophores and in size and shape of conidia, as illustrated by Miura (*13*), with *Cercospora daizu*. Nothing is yet known of the means of hibernation and dissemination of this fungus, but this is very probably accomplished by means of contaminated seed.

ANTHRACNOSE

Soy-bean anthracnose (*Glomerella glycines* (Hori) Lehman and Wolf), in its conidial stage on pods, was first collected in Chosen in 1917. It was named *Colletotrichum glycines* Hori, although Hori has not published the results of his studies. Its essential morphological features, however, are recorded by Hemmi (*7*) and his account contains appropriate drawings of acervuli, conidia, and setae. To date, the writers have not been able to learn of its occurrence in other countries in eastern Asia, nor have they seen specimens from the Orient.

Anthracnose was found in North Carolina for the first time in the summer of 1920. It was collected on both stems and pods, on which it forms numerous black acervuli which accord well with the descriptive account of *Colletotrichum glycines* by Hemmi (*7*). The perithecial stage was developed in culture the first season the disease was studied, but was not found in nature until the spring of 1924.

Since the writers' investigations (*12*) have shown that diseased seed serve as a means for the dissemination of the disease it is to be expected that it will be found to occur in other States where this crop is grown.

BACTERIAL BLIGHT

Several organisms have been found to be associated with bacteriosis of soy beans, as has been briefly discussed in a recent paper (*18*). The disease to which the name bacterial blight has been applied is caused by two easily distinguishable organisms, *Bacterium glycineum* Coerper, and *Bact. sojae* Wolf. No diagnostic features have yet been noted, however, for differentiating between the diseases which these organisms produce.

The writers recently received from Hemmi specimens of a bacterial disease collected in June, 1914, at Suwon, Chosen, by Takimoto. These specimens are some of those of the collection upon which Takimoto based his investigations (*16*). The organism which he isolated from this collection was not assigned a name by him but was compared with the published account of *Bacterium glycineum* (*2*), that of *Bact. sojae* (*15*, *17*), and that of *Pseudomonas glycineum* Nakano, and found to be most like *Bact. sojae*. He further pointed out that the leaf lesions are quite like bacterial blight in America, but that the occurrence of dark, sunken areas on petioles and stems are features not found associated with bacterial blight in North Carolina. In the writers' opinion, Takimoto's specimens present the appearance of characteristic leaf lesions of bacterial blight. In the light of the fact that Takimoto concluded that the organism which he was studying differed from *Bact. sojae* only in the absence of a capsule, in its failure to effect a change in milk, and in its ability to grow in the closed arm of fermentation tubes, differences which might not be manifest if cultures of the organisms from Chosen and from North Carolina were in the hands of one investigator, and that

Takimoto's specimens look like bacterial blight in North Carolina, it is the writers' belief that the diseases are identical.

The writers have not seen the paper by Nakano in which he gives an account of his studies on *Pseudomonas glycineum*, but Hemmi sent them a copy of Nakano's manuscript. Nakano isolated this organism from collections of a leaf-spot disease found in Kiushu, Kumamoto. He compared it with *Bact. phaseoli* E. F. S., *Bact. viciae* Takimoto, and the soy-bean bacterial-blight organism. These studies show that the bacterium is rod-shaped, and motile by means of a single polar flagellum. It forms yellow to orange colonies on culture media, does not liquefy gelatin, does not form acid from sugars, nor does it curdle milk. On the basis of these characters Nakano concluded that it is distinct from the bacterial-blight organism.

GENERAL DISCUSSION

All of the diseases which have been discussed in the foregoing account, except wilt and Cercospora leaf spot, have been definitely proved to be seed-borne. They comprise all the known diseases of soy beans in North Carolina which are of economic importance, except two—bacterial pustule (*Pseudomonas phaseoli var.* sojense Hedges) and sclerotial blight (*Sclerotium rolfsii*). The former of these also has been found to be disseminated by means of the seed. Neither has been reported, however, from the Orient.

Soy beans in Manchuria are subject to attack by a fungus which has been identified as *Sclerotinia libertiana*. Singularly, this organism occurs on various crops in North Carolina, but it has not been noted to infect soy beans. Aside from these a number of other fungi have been found in Manchuria to be associated with soy-bean diseases, some of the most important of these being *Hypochnus centrifugus* Tul., *Uromyces sojae* (P. Henn) Syd., and *Pleosphaerulina sojaecola* (Massal.) Miura. whose conidial stage is *Phyllosticta sojaecola* Massal.

SUMMARY

It has been found that various diseases of soy beans are transmitted by means of the seed. Because of this fact it was to be expected that these diseases would occur in eastern Asia, where soy bean is native. To date the following diseases are known to be common both to the Orient and to North Carolina: Wilt (*Fusarium tracheiphilum* Smith); mildew (*Peronospora manshurica* (Naoum.) Syd.); brown spot (*Septoria glycines* Hemmi); pod-and-stem blight (*Diaporthe sojae* Lehm.); anthracnose (*Glomerella glycines* (Hori.) Lehm. and Wolf); Cercospora leaf spot (*Cercospora daizu Miura*); and bacterial blight (*Bacterium sojae* Wolf).

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